Volume LXXIII Number 3

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Editor: JOHN M. COULTER

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EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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INFLUENCE OF SALTS ON BACTERIAL ACTIVITIES OF SOIL

J. E. Greaves

Salts occurring naturally in soils and those applied to them in various operations influence the number, species, and activity of the soil microflora. These factors in turn are reflected by yields obtained. Some substances applied to a soil may serve as food for the growing plant; others increase plant growth indirectly. This latter effect may be due to the changing of the physical, chemical, or bacterial properties of the soil. The substance may alter the physical properties of the soil to such an extent that the bacterial flora is modified. This in turn may increase or decrease the available plant food of the soil. Other substances may react chemically with constituents within the soil, and in so doing liberate nutrients which may be utilized directly by the growing plant (**24**). Again, they may directly modify the microflora and microfauna of the soil both as to numbers and physiological efficiency. In some cases all three changes may be wrought by the same salt. In each case the soil is so changed that its crop-producing power is modified. The question arises, therefore, as to what effect this or that fertilizer or soil amendment is going to have upon the bacterial activity of the soil. Furthermore, there are millions of acres of land which contain varying amounts of soluble salts. Some of these soils contain such large quantities of the so-called "alkalis" that no vegetation grows upon them.

Other soils contain only a medium amount of soluble salts, and the vegetation consists chiefly of alkali-resisting plants. Still other soils contain much smaller quantities of soluble salts, and they become injurious only when the soil is improperly handled. The reclaiming of the heavily charged soils and the maintaining of the others in a productive condition are problems confronting the soil chemist. These problems can be solved more successfully when the laws governing the influence of salts upon the growing plant and their action upon the chemical, physical, and biological properties of the soil are understood. This study was undertaken, therefore, with the hope of obtaining light on some of these laws. It was carried on with soils which naturally were productive, but

TABLE I

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO RETARD AMMONIFICATION IN THE SOIL IN UNIT TIME

	Chloride	Sulphate	Nitrate	Carbonate
Calcium	156×10^{-7}	5×10^{-3}	78×10^{-7}	11×10^{-3}*
Potassium	625×10^{-7}	125×10^{-6}	1×10^{-3}	6×10^{-3}
Iron	125×10^{-6}	625×10^{-7}	25×10^{-5}	6×10^{-3}
Manganese	125×10^{-6}	25×10^{-5}	5×10^{-4}	11×10^{-3}
Sodium	125×10^{-6}	5×10^{-4}	5×10^{-4}	6×10^{-3}
Magnesium	5×10^{-4}	5×10^{-4}	156×10^{-7}	1×10^{-3}

* Not toxic at 11×10^{-3}, highest concentration tested.

became unproductive through the addition of known quantities of various salts. This would give a soil which at first had the same physical, chemical, and biological properties, hence any difference found must be due to the salt added.

Using as a measure of toxicity of the various salts that quantity which if applied to a definite weight of soil reduces the ammonia produced in the soil as compared with a similar untreated soil kept under similar conditions, the values reported in table I were obtained. The quantity recovered from the soil by leaching was also determined, but for this study the quantity applied is used for the reason that the direct or indirect action of the addition of a specific salt is being determined and not the absolute point of toxicity, which undoubtedly will vary with different soils and conditions.

The soil used was a calcareous loam (**8**), rich in potassium, phosphorus, and all the essential elements except nitrogen, which was low, as was also the organic matter of the soil. The results as reported are the average of a great number of determinations, and represent rather accurately the toxic point of the various salts in this specific soil, hence the results represent the relative toxicity for several salts in this specific medium. This may or may not vary in a different medium. The results, therefore, are not to be taken as absolute but as relative, which can only justly be comparable with other results obtained under like conditions.

It is quite evident from these results that the toxicity of a compound is governed by both anion and cation. Without exception the chlorides are more toxic than the corresponding nitrates. The sulphate varies, depending upon the cation with which it is combined, whereas in every instance the carbonate is less toxic than any of the other salts. The relative toxicity for the anions, therefore, can be written in the order $Cl > NO_3 > SO_4 > CO_3$, thus indicating that the monovalent anions are more toxic than the divalent anions. It must be borne in mind, however, that there would be more anions in unit volume of the monovalent than of the divalent, where a divalent cation is combined with the monovalent anion, as these salts were tested on the basis of mole concentrations. When re-examined with equivalent ionic concentration, the difference, although small, still maintained the same order.

Excluding the difficultly soluble salts, and averaging the results for the cation, we obtain the series $Mn > Mg > Fe > Ca > Na > K$, or the divalent cation is found to be more toxic than the monovalent. Here also the number of ions would enter, and if the toxicity be due either to an osmotic or precipitant reaction the order is what would be expected.

Examining the results obtained with nitrification (**13**) on the same basis as has been done with ammonification, we obtain as the points of toxicity the results given in table II.

The most important conclusion to be drawn from these results as compared with the preceding is the greater sensitiveness of the nitrifying organisms to the different salts as compared with the

ammonifying organisms. There are three chlorides, one nitrate, and one sulphate which were slightly more toxic for nitrifiers than for ammonifiers. In most cases the resistance of the ammonifiers to salts is sufficiently greater that nitrification in a soil may be materially decreased without seriously interfering with ammonification. Moreover, the toxicity of salt increases with concentration much more rapidly toward nitrifiers than toward ammonifiers.

TABLE II

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO RETARD NITRIFICATION IN SOIL IN UNIT TIME

	Chloride	Nitrate	Sulphate	Carbonate
Calcium	78×10^{-7}	156×10^{-7}	3×10^{-3}*	625×10^{-7}
Potassium	156×10^{-7}	312×10^{-7}	156×10^{-7}	156×10^{-7}
Iron	1×10^{-3}	312×10^{-7}	156×10^{-7}	625×10^{-7}
Manganese	312×10^{-7}	25×10^{-5}	1×10^{-3}	78×10^{-7}
Sodium	4×10^{-3}	156×10^{-7}	156×10^{-7}	156×10^{-7}
Magnesium	78×10^{-7}	78×10^{-7}	625×10^{-7}	78×10^{-7}

* Not toxic at 3×10^{-3}, highest concentration tested.

TABLE III

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO RETARD AZOFICATION IN SOIL

	Chloride	Sulphate	Nitrate	Carbonate
Sodium	2×10^{-3}*	2×10^{-3}*	2×10^{-3}*	2×10^{-3}*
Potassium	2×10^{-3}*	1×10^{-3}	2×10^{-3}*	125×10^{-6}
Calcium	1×10^{-3}*	1×10^{-3}*	1×10^{-3}*	1×10^{-3}*
Magnesium	1×10^{-3}*	1×10^{-3}*	1×10^{-3}*	1×10^{-3}
Manganese	1×10^{-3}*	1×10^{-3}	1×10^{-3}*	1×10^{-3}*
Ferric	5×10^{-3}*	5×10^{-3}*	5×10^{-3}	5×10^{-3}

* Not toxic at this concentration, the highest tested.

The order of toxicity of the anion in the case of the nitrifiers is nearly the reverse of that for the ammonifiers. The order for nitrification is $CO_3>NO_3>SO_4>Cl$, whereas the order of toxicity of the cation as measured in terms of nitrification becomes $K>Mg>Fe>Mn>Ca>Na$. It is evident, therefore, that both the anions and cations exert an influence in determining the toxicity of the salts.

Examining nitrogen fixation (**12**) in the same light the results given in table III are obtained.

Possibly potassium carbonate is more toxic to the nitrifiers of the soil than it is to the nitrogen-fixing organisms. It is certain that the azofiers in soil are much more resistant to these salts than are the other classes of microorganisms. The concentration of the salts in the soil was not high enough to permit conclusion as to the relative toxicity of the various anions and cations. The tests, however, make it certain that alkali soils which have a vigorous nitrifying or ammonifying microflora will maintain a vigorous azofying flora, even though they contain considerable quantities of soluble salts.

The influence of an anion upon the internal friction of colloids varies with concentration and reaction of medium, and it is interesting to note that the series for the ammonifiers is the same as that for acid solutions of proteins, whereas that for the nitrifiers is the order for these ions upon a neutral or alkaline solution of the protein. If, therefore, toxicity of these salts is due in a measure to their changing the internal viscosity of the protoplasm, we should have to assume a slight difference in the protoplasm making up the cell of the two groups of microorganisms, the one being electro-positive and the other electro-negative.

The order of toxicity of the cation to the ammonifiers is not far different from what would be expected if toxicity were due to a precipitation of the protoplasmic colloids. When the nitrifying series are examined in the light of the HOFMEISTER series, however, the potassium ion is found on the opposite extreme of the series from where it should be.

Whether this is due to analytical error or to the potassium ion being especially poisonous because of its changing the state of turgescence of the organic colloid is not clear. It would appear, however, that if the latter were the correct explanation, we might expect potassium to change positions in the ammonifying series. This would appear more reasonable, for both the ammonifiers and nitrifiers function normally in the same medium, and the same evolutionary forces have been at work bringing these organisms to their present condition.

The relative toxicity of the anions toward ammonifiers and the relative toxicity of the cation as measured in terms of both ammoni-

fication and nitrification point to the conclusion that toxicity is due in a large measure to osmotic influences. This conclusion is based on the assumption that the salts on passing into solution behave as they would in pure water, an assumption which is not warranted, for it is a well known fact that the addition of a salt to a soil causes an exchange of ions. This may either increase or decrease the total number of soluble particles in the soil solution, and hence correspondingly change the osmotic pressure of the soil solution. The logical procedure, therefore, is to determine the osmotic pressure (**14**) of the soil at the concentrations at which the various salts become toxic to the different classes of microorganisms. The results for such tests for the ammonifying series are given in table IV.

TABLE IV

OSMOTIC PRESSURE OF SALT-TREATED SOIL TOXIC TO AMMONIFYING ORGANISMS

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium	1.62	2.77*	1.96	8.41*	1.79
Potassium	1.53	1.89	1.75	9.05*	1.72
Calcium	1.43	1.53	2.45	1.61	1.75
Magnesium	1.62	1.68	1.88	1.80	1.75
Manganese	1.56	3.53*	1.78	2.04	1.79
Iron	2.34	2.71*	1.84	1.84	2.00
Average	1.68	1.70	1.94	1.84	.....

* Not counted in the average.

Sixteen out of the twenty-four salts become toxic when the osmotic pressure ranges between 1.43 and 1.96 atmospheres. The average for the cation shows a variation of from 1.72 to 2.00 atmospheres, and in all except the iron ranges between 1.72 and 1.79, a difference of only 0.05 of an atmosphere. The anion shows a variation from 1.68 to 1.94. The small variation in osmotic pressure among the various salts at which they become toxic indicates that the osmotic pressure is an important factor in determining toxicity.

We find that when the ammonia produced had been reduced by about 10 per cent, the average osmotic pressure of the soil solution was 2.55 atmospheres; when the ammonia was reduced 25 per cent, the osmotic pressure was 5.49 atmospheres; and when ammonia

produced was 50 per cent of normal, the osmotic pressure was 9.5 atmospheres. Table VI gives these data for both ammonification and nitrification.

The nitrifiers, therefore, are more sensitive to osmotic changes than are the ammonifying organisms, but in both cases the toxicity increases more rapidly than does the osmotic pressure. This is very rapid in the case of the nitrifiers.

TABLE V

OSMOTIC PRESSURE OF SALT-TREATED SOIL TOXIC TO NITRIFYING ORGANISMS

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium	4.25*	1.81	1.80	1.84	1.82
Potassium	1.53	1.67	1.75	1.49	1.61
Calcium	1.60	1.54	.....	1.54	1.56
Magnesium	1.81	1.59	1.88	1.79	1.78
Manganese	1.65	2.73*	2.22	2.01	1.96
Iron	4.94*	1.89	1.85	1.86	1.87
Average	1.65	1.70	1.90	1.85	.....

* Not counted in the average.

TABLE VI

REDUCTION OF PRODUCT	OSMOTIC PRESSURE TO REDUCE	
	Ammonification 1 per cent	Nitrification 1 per cent
10 per cent	0.255 atmospheres	0.197 atmospheres
25 per cent	0.199 atmospheres	0.117 atmospheres
50 per cent	0.190 atmospheres	0.088 atmospheres

The average for the anions in the case of the nitrifiers is within experimental error, the same as that for the ammonifiers. The average for the cation is slightly lower for the nitrifiers than for the ammonifiers, but quite similar in both series, thus indicating that the toxicity in both sets is governed by the same factor, possible osmotic pressure plus a physiological effect produced by the salt. The physiological influence may be due to the replacing of ion in the living protoplasm, thus changing its physical, chemical, and electrical properties so that they are incompatible with life. It is thus assumed with LOEB (18) that the toxicity of sodium salts on entering the cell is due to the formation of sodium proteinates,

which, if present in too great a proportion in the cell, confer upon the protoplasm properties which are incompatible with the maintenance of normal functioning. The toxicity of calcium salts is likewise attributed to an undue predominance of calcium proteinate in the cell. An admixture of several types of protein salts is required to give the protoplasm of the cell the exact qualities essential to the maximal furtherance of its vital activities. Two factors, therefore, may enter in the toxicity of a salt: (1) the permeability of the salt for the living protoplasm, and (2) the chemical, physical, or electrical influence of the salt upon the protoplasm after entering the cell.

Those cases in which the osmotic influence is the predominating factor should show a marked decrease in toxicity when the solution is diluted (11); whereas in those cases in which the physiological effect predominates, the addition of another salt which would increase osmotic pressure may show a decrease in toxicity, due to a physiological balancing of the solution.

Numerous experiments have shown that the relative toxicity of sodium chloride, sodium carbonate, potassium carbonate, and calcium carbonate, as measured in terms of ammonification, decreases as the water added to a soil increases. All the other salts become relatively more toxic, thus indicating that some factor in addition to osmotic pressure is entering. In this regard the nitrifying organisms act quite differently, and toxicity is neutralized with potassium chloride, potassium sulphate, magnesium nitrate, and magnesium chloride, but not with the remaining salts when water is added. Were osmotic pressure alone the disturbing factor, it would be impossible to neutralize the toxic action of one salt by the addition of another, thus increasing osmotic pressure and at the same time decreasing toxicity.

This conception of antagonism and balanced solutions was first applied to a study of bacteria by LIPMAN in 1909. In his experiments (20) on the rate of ammonification of *Bacillus subtilis*, he showed that there is some antagonism between sodium and magnesium. On the other hand, he (21) found no antagonism, but increasing toxicity, when magnesium and calcium were com-

bined. Later he (**22**) demonstrated that there exists, as measured by ammonification, a true antagonism between sodium chloride and sodium carbonate, and between sodium sulphate and sodium carbonate, thus indicating that the anions as well as the cations at times may play a part in antagonism.

KELLEY (**16**), in studying the ammonification and nitrification of certain soils, found no antagonism between magnesium and sodium. LIPMAN and BURGESS (**23**), however, observed in the case of nitrogen fixation by *Azotobacter chroococcum* an antagonism between sodium and magnesium.

WINSLOW and FALK (**39**) have observed antagonistic effects in experiments on *Bacillus coli*. They found that cultures suspended in solutions of sodium chloride or calcium chloride were decreased in number, that higher concentrations produced sterilization of the culture, and that a combination of sodium chloride and calcium chloride in the molecular proportions of 5 to 1 was favorable to the growth of the organisms.

SHEARER (**29, 30**) also demonstrated similar effects of salts upon the viability of *Meningococcus* and *Bacillus coli*. He found that a combination of sodium chloride and calcium chloride was favorable to growth, whereas each salt used separately retarded growth.

BROOKS (**1**) found that, as measured by the rate of respiration of *Bacillus subtilis*, there is a marked antagonism between sodium chloride and calcium chloride, and between potassium chloride and calcium chloride. The antagonism between sodium chloride and potassium chloride is slight, and the antagonism curve shows two maxima. Later, using the same method and organism, he (**2**) found a well marked antagonism between magnesium chloride and sodium chloride, and, contrary to the findings of LIPMAN (**21**), a very slight antagonism between magnesium and calcium. This is in keeping with my own (**9**) experience, which showed that a true antagonism exists between calcium sulphate *vs.* sodium carbonate, calcium sulphate *vs.* sodium nitrate, calcium sulphate *vs.* sodium sulphate, calcium sulphate *vs.* calcium chloride, calcium sulphate *vs.* magnesium sulphate, as measured in terms of ammonification.

This clearly indicates that the anion as well as the cation plays a part in antagonism. A similar antagonism exists between these salts as measured in terms of nitrification.

Furthermore, using the ammonia produced as the criterion, an antagonism is seen to exist between sodium sulphate *vs.* iron sulphate, calcium chloride *vs.* iron sulphate, sodium chloride *vs.* iron chloride, sodium chloride *vs.* iron sulphate, magnesium chloride *vs.* iron nitrate, sodium chloride *vs.* iron carbonate, calcium chloride *vs.* iron carbonate, calcium chloride *vs.* iron nitrate, sodium nitrate *vs.* iron chloride, calcium chloride *vs.* iron chloride, sodium carbonate *vs.* iron nitrate, sodium carbonate *vs.* iron carbonate, sodium sulphate *vs.* iron nitrate, sodium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron nitrate, sodium carbonate *vs.* iron sulphate, sodium nitrate *vs.* iron nitrate, sodium nitrate *vs.* iron sulphate, magnesium sulphate *vs.* iron chloride, and magnesium sulphate *vs.* iron carbonate. This was small in the case of the first pair, and increased in the order named until the last, which neutralized 75 per cent of the toxic effect of magnesium sulphate.

As measured in terms of nitrification, a true antagonism was found to exist between sodium carbonate *vs.* iron carbonate, sodium chloride *vs.* iron chloride, magnesium sulphate *vs.* iron nitrate, sodium carbonate *vs.* iron sulphate, sodium nitrate *vs.* iron sulphate, sodium sulphate *vs.* iron carbonate, calcium chloride *vs.* iron carbonate, sodium nitrate *vs.* iron carbonate, sodium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron carbonate, sodium nitrate *vs.* iron chloride, sodium sulphate *vs.* iron nitrate, sodium sulphate *vs.* iron chloride, magnesium chloride *vs.* iron carbonate, calcium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron chloride, sodium chloride *vs.* iron sulphate, magnesium chloride *vs.* iron chloride, sodium carbonate *vs.* iron chloride, and magnesium chloride *vs.* iron nitrate. This was low in the case of the first pair, and increased progressively in the order named up to the last named pair, in which the iron nitrate increased the nitrification 420.7 per cent over that soil treated with magnesium chloride alone.

The results, therefore, indicate the toxicity of soluble salts toward soil microorganisms to be due to an osmotic effect which makes it impossible for the cell to take up its normal nutrients,

but permits foreign or unbalanced constituents to enter. These foreign or unbalanced salts on entering the cell protoplasm interact with the proteins thereof, forming within the living protoplasm foreign proteinates, the physical, chemical, and electrical properties of which are different from those of the normal protoplasm. Hence we have the protoplasm rendered incapable of normal functioning. The first effect is governed to a marked extent by the osmotic pressure of the medium in which the organism is functioning, and the second by the specific salt, acid, or base which comes in contact with the protoplasm.

STIMULATING ACTION OF SALTS.—Many salts when added to a medium in which bacteria are functioning first stimulate, and as the concentration is increased the specific salt becomes toxic. The

TABLE VII

PERCENTAGES OF AMMONIA PRODUCED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium	106.0	107.8	100.0	110.1
Potassium	100.0	102.2	100.0	108.9
Calcium	100.0	100.0	103.2	114.6
Magnesium	100.7	100.0	104.5	103.5
Manganese	100.6	116.0	123.8	111.2
Iron	118.6	102.9	103.9	107.9

extent of this stimulation varies with the salt, the concentration of the salt, the medium in which it is used, and the specific micro-organism grown upon it. If the ammonia produced in unit time in untreated soil is taken as 100 per cent, we obtain the values given in table VII for the various salts.

All except six of the salts stimulate bacterial activity. There is a wide variation, however, depending upon the specific salt. The cations arranged in a descending order would be Mn>Fe>Na>Ca>K>Mg. Although there is a wide variation, depending upon the cation and anion, it is interesting to note that it is not these elements which are to be recognized as plant foods, but the catalizers which head the list. This also appears in the case of the anion where the series is $SO_4>Cl>CO_3>NO_3$. The cations would appear to play a greater part as bacterial stimulants than do the

anions. This indicates a very marked acceleration of the speed with which the protein is transferred into ammonia, which would result in a greater available supply of this compound for the action of the nitrifiers. The speed with which the ammonia is oxidized to nitric acid also increases with the addition of various salts, as is brought out in table VIII.

TABLE VIII

PERCENTAGES OF NITRIC NITROGEN PRODUCED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium	142.0	101.0	100.0	100.0
Potassium	106.5	106.4	100.0	100.0
Calcium	100.0	102.1	196.7	100.0
Magnesium	123.2	106.5	101.2	140.7
Manganese	112.9	125.4	113.2	108.4
Iron	128.4	100.0	102.0	117.4

TABLE IX

PERCENTAGES OF NITROGEN FIXED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium	102.6	102.7	104.4	107.3	104.2
Potassium	100.0	101.3	105.0	100.0	101.6
Calcium	167.6	109.9	102.6	103.3	104.0
Magnesium	102.2	101.9	101.7	101.9	101.9
Manganese	102.3	102.2	102.4	100.0	101.7
Iron	104.3	102.0	100.4	101.8	102.1
Average	101.9	103.3	102.7	102.3	.....

All but six of these salts increase the accumulation of nitrates in the soil. Two which did not stimulate the ammonifiers also failed to stimulate the nitrifiers. In four cases there was no agreement between ammonifiers and nitrifiers. The manganese and iron salts are both strong stimulants, as was the case with the nitrifiers; potassium, which is an essential element, is the least effective of the cations, whereas chlorine is one of the most active, being exceeded in activity only by the sulphate ion.

It is evident that the nitrogen available to higher plants would be very materially increased through the addition of salts to a soil,

and furthermore, it is usually those salts which are considered soil amendments that exert the greatest influence. What effect would these salts have on the total nitrogen of the soil? This is answered in table IX.

Again potassium is least efficient, while sodium is one of the most efficient. It is interesting to note that the nitrates, which are usually stated as retarding azofication, stimulate it to a greater degree than any of the other compounds. In this respect calcium nitrate is more efficient than any other salt. This probably indicates that it is best to add nitrates to the soil in this form. It could not be claimed that this compound was carrying available calcium to the soil, for this soil already contains some 40 per cent of calcium and magnesium carbonate.

It is evident that these salts would increase both total and available nitrogen in the soil. They would also increase the crop-producing power in another way, namely, by increasing both directly and indirectly the available potassium and phosphorous of the soil (10).

SOLVENT ACTION OF BACTERIA.—BROWN (3) found that twelve out of twenty-three bacteria isolated from soil exerted a definite solvent action on difficultly soluble plant food. One organism which produced no gas, but a large amount of acid, exerted the greatest solvent action upon calcium carbonate; while other organisms which produced gas (largely carbon dioxide), but not as much acid as the former, gave an action more marked than of the stronger acid producer upon the dicalcium and tricalcium phosphates. *Bacillus subtilis*, *B. mycoides*, *B. proteus vulgaris*, and *B. coli communis*, as well as several agar cultures from garden soil, were found (26) to be capable of dissolving the phosphates of bone and to a less extent those of mineral phosphates. The greatest solvent action was exerted in media containing sodium chloride, potassium sulphate, and ferrous sulphate. Even yeast (17) may play an important part in dissolving phosphates. KROBER, however, considers that the life activity of the bacteria, that is, assimilation of phosphorus by the living organism, plays little or no direct part in solution of the phosphates, but that the latter is due to the action of the organic acid and of the carbon dioxide produced.

The acids produced by bacteria act upon various phosphates, changing them to the soluble monophosphate, but the rate of solution varies widely with the different compounds. Tricalcium phosphate, in precipitated form, dicalcium phosphate, and tetracalcium phosphate of Thomas slag are much more rapidly dissolved than the crystalline or the so-called amorphous phosphates. The general reaction is as follows: $2RCOOH+Ca_3(PO_4)_2=Ca_2H_2(PO_4)_2+(RCOO)_2Ca$. The reaction takes place most rapidly in soils containing large quantities of organic matter due to the active fermentation taking place in such soils.

GRAZIA (**6**) considers enzyme action to play a part in the dissolving of phosphates in soil, for he found the addition of chloroform to a soil reduced bacterial activity and decreased the acid produced, but at the same time the solution of phosphates was increased. This is in keeping with the finding of BYCHIKHIN and SKALSKI (**4**).

The presence of ammonium chloride and sulphate in the cultural media is especially effective, according to PEROTTI (**25**), in increasing the solvent action of bacteria for phosphorus. PEROTTI considers the successive steps in the solution or decomposition of phosphorus in bacteria cultures to be as follows: (1) generation of acids, (2) secondary reactions in the solution, and (3) production of a soluble phosphorus containing organic substance. The first two of these are the result of the activity of the bacteria on the phosphorus, and the last is due to the metabolic assimilation of the microorganisms.

The oxidation of sulphur by soil bacteria at times may generate sufficient acid to play a very important rôle in the dissolving of soil phosphorus. HOPKINS and WHITING (**15**), however, consider that the nitrosomonus are of first importance in rendering phosphorus and calcium soluble, due to the nitrous acid produced from ammonia: $(NH_4)_2CO_3+6O=2\,HNO_2+H_2CO_3+2\,H_2O$. The resulting nitrous acid reacts with the raw rock phosphate rendering it soluble, thus: $Ca_3(PO_4)_2+4HNO_2=CaH_4(PO_4)_2+2Ca(NO_2)_2$.

Analyses showed that about one pound of phosphorus and about two pounds of calcium are made soluble for each pound of nitrogen oxidized, aside from the action of the acid radicles asso-

ciated with the ammonia. The carbonic acid would play an important part also in this reaction: $4H_2CO_3+Ca_3(PO_4)_2=2\ Ca(HCO_3)_2+CaH_4(PO_4)_2$. They found that neither ammonifying bacteria nor nitrobacter liberated appreciable quantities of soluble phosphorus from insoluble phosphates. Whereas this would readily occur in soil poor in calcium carbonate, yet in those rich in calcium carbonate there would be only small quantities of phosphorus liberated. This is the conclusion reached by KELLEY, but where the soluble phosphorus is rapidly being removed by the growing plant, there is little doubt but that the various soil organisms play an important part in rendering phosphorus soluble.

Moreover, it is quite evident that *Azotobacter* in their metabolism transform soluble inorganic soil constituents either into soluble or insoluble organic forms. This is especially true of phosphorus which is found in the ash of these organisms in such large quantities. The phosphorus, on the death of the organism, is returned to the soil in a readily available form, for STOKLASA has found that 50 per cent of the nitrogen of these organisms is nitrified within six weeks, and there is no reason for believing that the phosphorus would be liberated more slowly. There is also the possibility that many of the constituents of the bacterial cell may become available through the action of autolytic enzymes without the intervention of other bacteria (**19**).

It is further evident that an organism which possesses the power when growing under appropriate conditions of generating 1.3 times its own body weight of carbon dioxide during twenty-four hours (**34**) must greatly change the composition of the media in which it is growing. Water charged with carbon dioxide is a universal solvent, and will attack even ordinary quartz rock. Granite and rocks related to it are rather quickly attacked with the liberation of potassium and other elements. Likewise, it would act upon the tricalcium phosphate of the soil with the formation of more readily soluble phosphates, for this substance is four times as soluble in water charged with carbon dioxide as it is in pure water: $Ca_3(PO_4)_2+2CO_2+2H_2O=Ca_2H_2(PO_4)_2+Ca(HCO_3)_2$. Moreover, the nitrogen-fixing organisms produce, among other products, formic, acetic, lactic, butyric, and other acids. The

kind and quantity of each depend upon the specific organisms and upon the substance on which they are acting. These substances are sure to come in contact with some insoluble plant food which may be rendered soluble, for they have a high solvent power for the insoluble phosphates (**32**). The resulting salts of calcium would be attacked further by bacteria with the formation of calcium carbonate (**5**).

Whether these processes will give rise to an increase in the water-soluble plant food of the soil will depend upon whether the products of the second, the analytical reactions, exceed the products of the first, the synthetic reactions. We must not lose sight of the fact that although many of the organic phosphorus constituents may not be soluble in pure water, they may be more available to the living plant than are the constituents from which they at first were derived through bacterial activity. This being the case, variations may be expected in the results reported from laboratory tests. STOKLASA (**33**) found that bacterial activity rendered the phosphorus of the soil more soluble, whereas SEVERIN (**27**) in his early work found the opposite to be true. Others have found that the solvent action of bacteria for insoluble phosphates is in direct proportion to the acid secreted by the organism (**26**).

In a later work SEVERIN (**28**) obtained different results. He used three soils, one sterile, a second sterilized and inoculated with pure cultures of *Azotobacter*, and a third sterilized and inoculated with cultures of *B. radicicola* and *Azotobacter*. The solubility of the phosphorus increased 8 to 14 per cent over that in the sterile soil. The acid-producing organisms, due to the acid secreted and their intimate contact with the soil particles, possess the power of dissolving silicates. Moreover, arsenic greatly stimulates nitrogen fixation, and there is a relationship between this increased bacterial activity and the form and quantity of phosphorus found in such a soil (**7**).

Although the metabolic activity of *Azotobacter* gives rise to large quantities of phosphate solvents, these organisms transform phosphorus into organic phosphorus compounds less rapidly than do the ammonifiers (**35**). There are cases, however, in which bacterial activity has decreased the water-soluble phosphorus of

the soil and of raw rock phosphate (**36, 37**). This, however, does not indicate that it is less available, for, as pointed out by TRUOG (**38**), the mixing of floats with manure caused an immediate decrease in the solubility of the phosphorus in 0.2 per cent citric acid solution, yet when thoroughly mixed with the feeding area of the soil its availability was increased to such an extent that some species of plants apparently were able to secure almost an adequate supply of phosphorus from this material. The addition of manure to the soil greatly increased the carbon dioxide production, and for a short time measurably increased the solvent action on floats. Where there is a decrease for a time of water-soluble phosphorus in fermenting media, it is probably due to the formation of phosphoproteids within the bodies of the bacteria (**31**), and these would later be rendered soluble, due either to further bacterial activity or autolytic enzymes. This increased bacterial activity should and actually does result in an increased water-soluble and organic phosphorus of the soil, as may be seen from table X, in which the water-soluble phosphorus plus the organic phosphorus in the untreated soil has been taken as 100 per cent.

TABLE X

TOTAL WATER-SOLUBLE PHOSPHORUS PLUS ORGANIC PHOSPHORUS IN SOIL TO WHICH VARIOUS SALTS HAD BEEN APPLIED AND LEFT FOR THREE WEEKS, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium	124.0	99.8	.....	105.3
Potassium	106.6	105.7	.....	102.1
Calcium	101.6	105.9	88.4	99.1
Magnesium	103.1	105.3	108.4	85.2
Manganese	99.3	86.1	101.1	135.1
Iron	100.2	103.4	118.3	92.5

In all except seven cases where the salts had increased bacterial activities there also resulted an increase in the available phosphorus. Moreover, associated with this increase of available phosphorus go increased crops as found in field and pot experiments, and the significant feature of these facts is that there is also an increase in the phosphorus of these plants (**7, 10**).

Summary

Many salts when applied to a soil in small quantities increase the bacterial activities of that soil. This is manifest by an increased production of ammonia, nitrates, and soluble and organic phosphorus, together with an increased nitrogen fixation. Usually, although not always, those salts which become toxic in the lowest concentration are the greatest bacterial stimulants.

There is a very close correlation between toxicity of the various salts and the osmotic pressure produced in the soil, thus showing that toxicity is due in part to osmotic disturbances. Another factor of equal importance is the change in chemical composition of the protoplasm resulting from the formation of salts of the protein other than those normally occurring in the living protoplasm, thus incapacitating them for their normal functions.

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VASCULAR ANATOMY OF ANGIOPTERIS EVECTA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 290

HUGO L. BLOMQUIST

(WITH PLATES V–VIII AND EIGHT FIGURES)

In the study of the vascular anatomy of plants it has been realized that it is necessary to study the successive stages which the plant passes through in the progress of its development. Since it is the aim, first of all, to determine the position, interrelationship, and structure of the different vascular components of the mature plant, this procedure not only facilitates the work, but in most cases perhaps is absolutely necessary. Likewise, in the search for facts which are to show indications of phylogenetic relationship, it is unnecessary to point out that it is in the developmental stages that these are most likely to be found. This method has proved to be especially necessary in the case of the Marattiaceae, for the vascular structures in the mature plant are not only very complex and difficult to interpret, but as the plant develops there are marked changes appearing in the anatomical structures from stage to stage. In some of the previous investigations on the anatomy of the Marattiaceae the failure to follow this method has led to much confusion and incorrect interpretation of the facts.

Historical

Considerable attention has been given to the Marattiaceae in the last fifty years, due chiefly to the evidence from geological records that this group of ferns, or a closely related group, was of great abundance and wide distribution in the past, and to the unique morphological and anatomical features which this group presents. Most of the work done on the anatomy of the Marattiaceae has been rather scattered and incomplete. The reason for this has chiefly been the lack of material, as these ferns are restricted to rather inaccessible parts of the world. Although plants have been transported to many conservatories, this has not

appreciably altered the situation, because of the tardiness in the germination of spores and the slowness of growth of the gametophyte.

The investigations on the anatomy of the Marattiaceae up to 1900 have been reviewed by Miss SHOVE (**12**). It is evident that the work up to that time was rather unsatisfactory, as only mature stems were dealt with, and the material in most cases was limited to one specimen. Miss SHOVE also worked with a mature stem. Since then, however, several investigations have been carried out in which the younger stages have been studied. In 1902 FARMER and HILL (**8**) published the results of an investigation on the development of the vascular anatomy in *Angiopteris evecta*, which was supplemented by a paper on the younger stages by CAMPBELL (**2**) in 1909. A thorough investigation on the development of the vascular anatomy of *Marattia alata* was carried out by Miss CHARLES (**7**) in 1911. This was the first investigation on the anatomy of this group based on an abundance of material. In 1917 WEST (**13**) added another contribution to the knowledge of the vascular anatomy of this group in a paper on the anatomical structures of *Danaea* spp. and several of the other genera. This investigation also was based on an abundance of material.

In comparing the conclusions drawn in this literature, it is evident that the vascular anatomy of the plants of the six genera of the Marattiaceae is very complex, but shows a striking similarity in facts both in the developmental stages and the more mature plants. Differences which are reported are mainly in details and in the interpretation of facts. There seems to be an agreement of opinion that the sporophyte of the Marattiaceae is traversed by a central strand of vascular tissue, called a dictyostele, from which roots and leaf traces arise. This stele is at first solid, or protostelic, then passes to a more or less tubular form, a solenostele, which is soon broken open by leaf gaps which are at first wholly repaired, but later overlap, so that the central strand becomes a banded structure which appears crescent-shaped in cross-section. This at first is simple, but later becomes complicated by the appearance of commissural and medullary strands. Disagreement is shown in regard to the meristematic regions, the

presence or absence of an endodermis, the origin of lateral roots, and whether the central strand is of cauline or foliar origin. The relationship between roots and leaves and the vascular arrangements in the stipules are also points still unsettled.

Material and methods

The material used in this investigation was collected on the island of Tutuila, Samoa, by Dr. W. J. G. LAND, of the Hull Botanical Laboratory, during October and November 1912. It consisted of an abundance of young sporophytes of *Angiopteris evecta*, ranging from the first to the tenth leaf stages and a few somewhat more advanced.

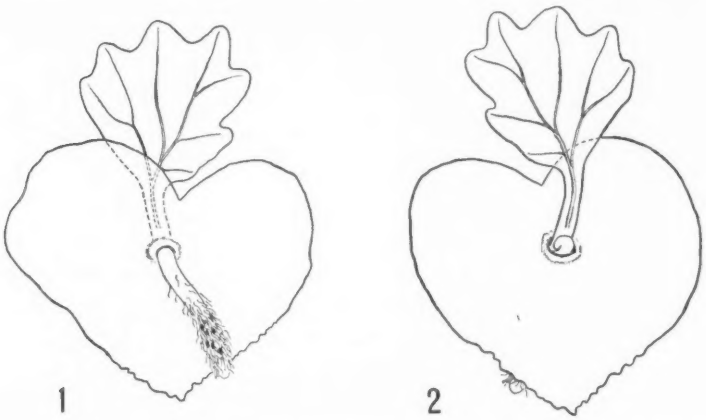
This material was imbedded in paraffin, and complete serial sections ranging from 10 to 20 μ in thickness were made with a rotary microtome. These sections were then studied from below upward, and diagrammatic reconstructions were drawn of the different stages. From these a final reconstruction was made in which the different stages were superposed one upon another to show as clearly as possible the successive stages which the plant passes through in the progress of its development. The most serious difficulty encountered was the fact that as the sporeling develops beyond the third and fourth stages, the lower part of the stem becomes more or less distorted, and in still older stages even decays. This difficulty was overcome by studying a close series of stages, which the material provided, selecting characteristic sections from the younger stages, and carefully matching these with sections representing the same morphological location in the older stages. The possibility of misinterpretation due to local variations, which are quite common in the anatomy of *Angiopteris*, was guarded against by studying several specimens of the same or approximately the same stage.

Investigation

EXTERNAL FEATURES

While the external features of *Angiopteris* have been described by several investigators, these will be reviewed in part to assist in relating the internal structures with the external features. The

first peculiarity of the young sporophyte of *Angiopteris*, as well as of all Marattiaceae, is the way in which the first leaf makes its exit from the gametophyte. As is well known, in most ferns the primary leaf grows first downward, and then emerges from under the gametophyte through the notch in the heart-shaped form of the latter. In *Angiopteris*, on the contrary, the first leaf pushes its way upward through the gametophyte and emerges on the dorsal side, as shown in figs. 1 and 2. CAMPBELL (3) attributes this behavior to the fact that the first division of the fertilized egg comes



FIGS. 1, 2.—Gametophyte and sporeling of *Angiopteris*, showing mode of exit of primary sporophyte organs from prothallus; ×4.

in transversely to the axis of the archegonium, or parallel with the axis of the gametophyte; while in most ferns it is usual to find the first wall perpendicular to the gametophyte, or parallel with the axis of the archegonium. In an unpublished paper by LAND (11) on the embryogeny of *Angiopteris*, the presence of a well defined suspensor is reported, and he suggests that this organ very probably is responsible for the initial upward growth of the primary leaf in the Marattiaceae. The suspensor has now been reported for two other genera, *Macroglossum* (5) and *Danaea* (1), and it is probable that its presence in the remaining genera awaits further investigations.

The next point to be observed is the appearance of the stem tip. About the time the first leaf has emerged from the gametophyte, a slight depression is seen on the dorsal side of the petiole where this passes into the root. At the bottom of this depression there is a well defined apical cell, which can easily be seen from longitudinal sections of the young sporophyte. The second leaf appears on the crest of this depression, usually about 130° from the first, while the corresponding root emerges directly below, next to the primary root. The succeeding leaves appear at approximately the same distance from the next older leaf that the second appeared from the first, thus forming a spiral arrangement which may be clockwise in one plant and counter-clockwise in another. This habit has an important bearing upon the development of the internal structure, as will be shown later. The succeeding roots grow through the cortex and appear from below the thickening stem, and later penetrate the sides. The relation between the roots and the corresponding leaves cannot be determined from external appearances after the third and fourth leaves have appeared. In none of the specimens studied had the roots branched, and no differences, except in size, could be discerned from the external appearances of the different roots.

A characteristic feature of the mature plants of the Marattiaceae is the presence of conspicuous fleshy stipules. In the first leaf of *Angiopteris* no stipules are visible, while in the second slight thickenings show their rudiments. No well developed stipules appear until the fourth leaf. At this stage the stipules appear as lateral swellings on the petiole as soon as the leaf is visible. As the leaf develops and the petiole elongates, the upper part of the stipule splits away from the petiole, and this gives rise to the more or less pointed lobes. In all sporelings examined there were no indications of dorsiventrality, either in external appearance or internal structure.

General internal structure

The general internal structure of a stem of *Angiopteris* which has developed beyond the first few stages may be divided into two main regions, the cortical and the central vascular. The cortex,

which is composed of rather large, thin-walled cells without intercellular spaces, is relatively thick, with an abundance of starch deposited in the cells surrounding the central region. In the younger stems cells filled with tannin are quite abundant, but in the older stages these are less common. This cortical area is traversed by the outgoing leaf traces, and numerous roots digest their way down through it. The leaf traces are single in the younger stages, but in the older levels these bifurcate before entering the stipules. Because of the spiral arrangement of the leaves, the leaf traces are arranged in zones which exhibit a spiral appearance in cross-section, in which the younger are closer to the center than the older. No fusing of leaf traces or a branch from one leaf trace with that of another takes place. The leaf traces pass outward directly.

The central region consists of the vascular tissue, imbedded in a ground tissue which may be called the central parenchyma. This parenchyma does not differ markedly from the cortical tissue except that the cells are somewhat smaller, have thinner walls, and less starch. In the first few stages there is a conspicuous endodermis, but in the older stages there is no such layer of cells which marks off the central region from the cortex, and the central parenchyma passes insensibly into the former. The central vascular tissue consists of a strand which appears more or less crescent-shaped in cross-section. The xylem is endarch and is completely surrounded by phloem. From one edge of this strand leaf traces are given off, and this contribution is always from the same edge in the same plant (figs. 3, 4). On the outer side of the other edge of this central strand a root stele is joined, usually at about the same level that the leaf trace is given off. The vascular tissue thus contributed to the leaf traces is made good on the other edge in two ways, by an increase of vascular tissue on the edge itself and by the addition of commissural strands. From the edge of each leaf trace abutting on the opening in the central strand a commissural strand is given off where the leaf trace is freeing itself from the central strand. This bends outward and passes diagonally upward and fuses with the opposite edge of the central strand just below where the next root above is attached (figs. 9–15).

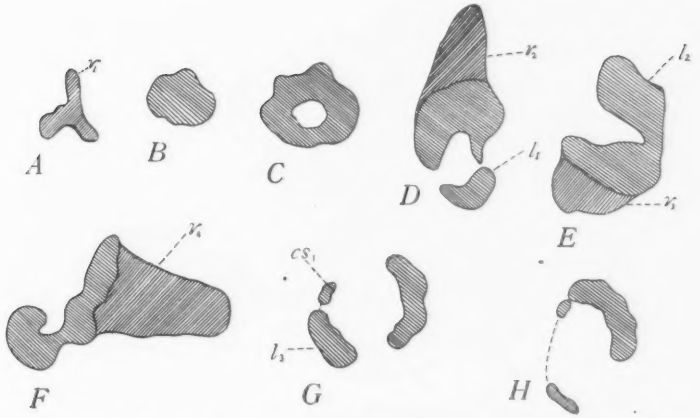
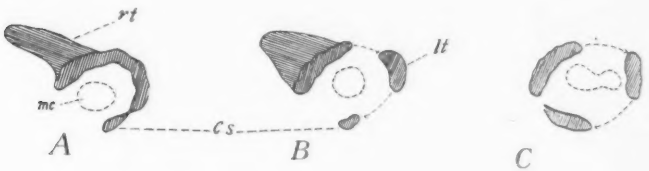


FIG. 3.—Diagrams of sections of stele, showing stele development up to third leaf (only xylem shown); sections not successive: r_1, r_2, r_3, r_4, first, second third, and fourth root traces; l_1, l_2, l_3, first, second, and third leaf traces; cs_1, first commissural strand; ×176.



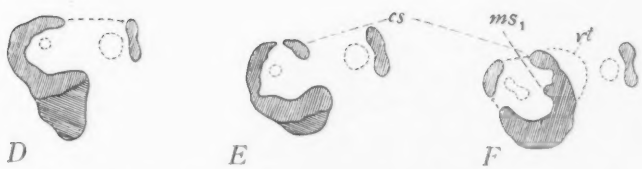


FIG. 4.—Diagrams of sections of stele, commencing slightly below where first medullary strand appears (only xylem shown); sections not successive: *rt*, root stele; *cs*, commissural strand; *lt*, leaf trace; *mc*, mucilage canal; ms_1, first medullary strand; ×80.

It is evident that by a continual loss of tissue from one edge and an addition on the other, in the successive sections, the crescents will appear to move in a circle, indicating that the strand is a more or less open spiral. The explanation for this spiral condition is to be found in the spiral succession of the leaves. In the stages above the eighth leaf, a medullary strand appears on the inner side of the central strand, usually slightly above and opposite the junction of a root stele. This passes diagonally upward, crosses the central parenchyma tissue, and fuses with the commissural strand before the latter has joined the central strand (fig. 5). In this condition they meet the edge of the central strand, and the commissural strand fuses with the latter and is lost. The medullary strand, fused to the inner side of the central strand, but retaining its identity, passes upward until the union of the root and the central strand has been cleared, when it frees itself and repeats its course across the central parenchyma tissue. This behavior of the medullary strand agrees in the main with the condition in *Marattia* as described by Miss CHARLES (7). In the lower levels of the stem a mucilage canal appears in the center of the parenchyma tissue. This canal divides wherever a leaf trace is given off, and one of the branches follows the inner side of the latter.

Each leaf trace is definitely related to one root. HOLLE (10) has reported the same condition in *Marattia*, but found two roots to each leaf in *Angiopteris*. The writer in some cases found two roots attached on the same level, and it appeared as if two roots were related to one leaf. After following out the series, however, it was found that these roots were related to different leaves. This variation will be discussed more fully later. The root which appears almost opposite each leaf trace is related to the next leaf above. In the younger stages each leaf trace is joined to the central strand directly above the insertion of its corresponding root (fig. 9); but in the older stages the leaf trace may be displaced to the right or left of the corresponding root, as the case may be, due to the spiral condition of the central strand (figs. 11–15). The distance between the root stele and the junction of the corresponding leaf trace above increases also in the successive stages.

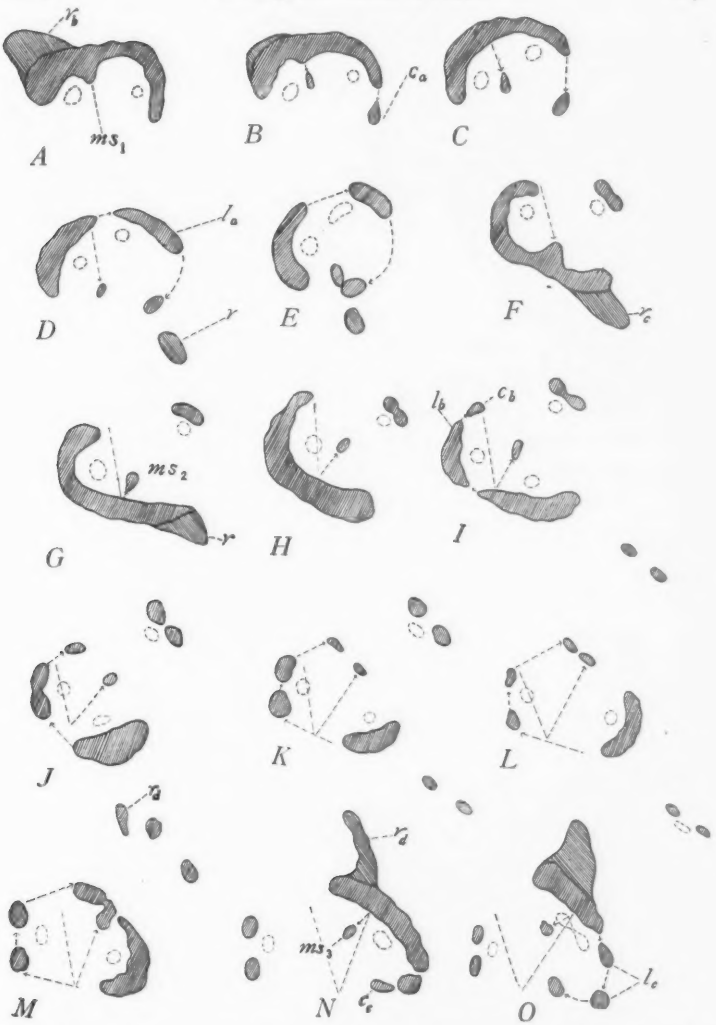


FIG. 5.—Diagrams of sections of stele, showing course of different vascular components after appearance of medullary strand (only xylem shown); sections not successive: *r*, root stele; *l*, leaf trace; *ms*, medullary strand; *c*, commissural strand; *mc*, mucilage canal; dotted lines with arrows indicate origin; and approximate courses of leaf traces, commissural strands, and medullary strand; ×16.

First leaf stage

The first few stages have been described by Farmer (**8**) and Campbell (**2**), and the present investigation adds little to their descriptions. The primary root is stated by them to be diarch. The writer found this to be true in some cases, but in as many cases the root was found to be triarch. Similar variations have been reported by Campbell in *Danaea*, *Botrychium*, and *Helminthostachys* (**3**). The endodermis is well organized and is separated from the protoxylem and protophloem by a uniseriate pericycle. In some places the protoxylem abuts directly on the endodermis, as has been reported previously (**8**). In the diarch stele there are two protophloem points and in the triarch three (figs. 16, 17).

The transition region in *Angiopteris*, as reported for other Marattiaceae, is a solid strand of vascular tissue with more or less irregular outline (fig. 19). In passing from this region upward, some cells in the xylem remain unlignified, usually near the center of the stele, so that the latter may become more or less tubular in cross-section. This condition has been called a solenostele (fig. 25). In some cases, however, these parenchyma cells are more scattered, and the condition resembles what has been reported for *Danaea* by Brebner (**1**). In this genus the parenchyma cells appear without any relation to the center of the protostele. In either case the appearance of these unlignified cells marks the union of the next root stele and the corresponding leaf trace above. The writer agrees with Brebner that "the parenchyma . . . . called pith is probably simply due to the root-junction and the preparation of the departure of the leaf trace." No phloem is developed in the center of the tubular stele at this stage.

A longitudinal section of the sporeling at this stage shows a well defined apical cell at the stem tip (fig. 18). This apical cell has been reported by Campbell in *Macroglossum* (**5**), by Miss Charles in *Marattia* (**7**), and by West in *Danaea* (**13**). In a transverse section it was impossible to locate definitely this apical cell, but from the shape of the cells in the stem tip region it seems that this cell is four-sided. No procambial tissue is to be observed below the stem tip, a condition which has been reported by Brebner for *Danaea* and by Campbell for *Angiopteris*. The first differentia-

tion of vascular tissue is found to be related to the second leaf trace. The few tracheids which join up the first leaf stage with the second pass into the second root stele, while some pass directly upward and add to the structure of the second leaf trace (fig. 3 *D*, *E*).

Second leaf stage

The second root appears slightly above the transition region, about 130° from the first leaf trace. Fig. 20 shows this root in a very young stage. It is impossible to state from what tissue this originates, since it appears before any differentiation of tissues has taken place. The same is also true of *Marattia*. The second leaf trace appears directly above the second root. Some variation occurs at this stage; the second leaf trace may be joined to the third by both edges, in which a second tube is formed and the gap formed by the first leaf is repaired. In as many cases, however, only one edge is joined up with the third leaf trace, and in such cases the leaf gap is not repaired. In either case the phloem occurs on the inside as well as on the outside of the vascular tissue. The endodermis which was very evident in the first stage is more difficult to locate. Although endodermal thickenings occur, the characteristic organization is more or less broken up. In no cases was the vascular tissue observed to become a solid strand after the junction of the first and second leaf stages had been passed. In *Marattia*, on the contrary, the protostelic condition may appear more than once.

Third leaf stage

After the second leaf stage has been passed, the vascular tissue of the central region never appears as a tube. The third root stele originates on the level with the outgoing of the second leaf trace from the central region. At this stage the leaf trace comes off from one edge of the central strand, which is the vascular tissue leading up to the fourth leaf trace (fig. 3 *F–H*). The latter tissue now assumes a crescent-shaped form in cross-section. A few scattered tracheids may come off the free edge of the third leaf trace and wander back toward the central strand, or may end blindly before arriving there. These tracheids represent the rudimentary commissural strand, which in many cases is fairly

well developed at this stage (fig. 3 *G*, *H*). The leaf trace passes out to the petiole undivided, and the stipules, which are not conspicuous at this stage, have no vascular strands.

Fourth and fifth leaf stages

In the fourth leaf stage the commissural strand is well developed and separates from the leaf trace after the latter has passed a considerable distance from the central strand, then it approaches the opposite edge of the latter. This behavior is the same as shown in fig. 4 *A–C*. Meanwhile this edge of the central strand is approaching the commissural strand by the addition of vascular tissues, and when the two meet, fusion takes place (fig. 4 *C–F*). At this stage a mucilage canal appears in the center of the central parenchyma. This divides where the leaf trace goes outward, and one branch follows the inner side of the trace. The fifth stage is very much like the fourth except that the strands are larger and the commissural strand leaves the leaf trace closer to where the latter comes off the central strand. The central strand has increased in size so that the root stele reaches only half way around the other side. No well defined endodermis is discernible in this and the subsequent stages.

Sixth and seventh leaf stages

The sixth and seventh leaf stages differ from the fifth mainly in the stipular region, which will be discussed later.

Eighth and ninth leaf stages

In the eighth leaf stage a new feature presents itself in the appearance of a medullary strand. This strand is first visible coming off the inner side of the central strand opposite the junction of the ninth root (fig. 4 *F*). The medullary strand takes a diagonally upward course, crosses the central parenchyma, and fuses only partially with the commissural strand immediately before the latter has met the central strand. Both join the edge of the central strand just below the junction of the tenth root (fig. 5 *A–F*). The commissural strand becomes an integral part of the central strand, but the medullary strand remains visible, joined to the inner side of the central strand, and passes upward to the upper level of the

root junction (fig. 14). In these stages the leaf trace bifurcates while still within the cortex.

Tenth and subsequent stages

The tenth stage is very similar to the eighth and ninth, except that the different strands have increased in size. In cross-section the crescent shape of the central strand is less evident, especially

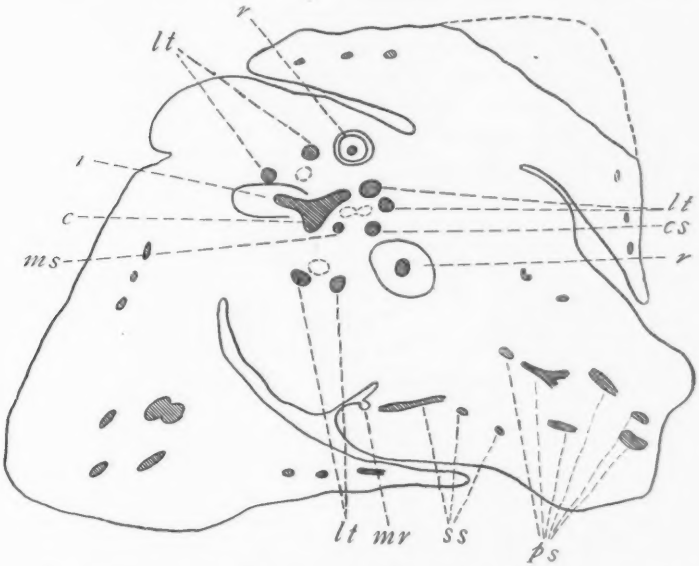


Fig. 6.—Diagram of section of stem above tenth leaf stage: *c*, central strand; *ms*, medullary strand; *cs*, commissural strand; *ll*, leaf trace; *ss*, stipular strands; *ps*, petiolar strands; *r*, root; *mr*, meristematic region of stipule; ×8.

in the region of a root junction. In some places the commissural strand and the central strand are very similar both in size and shape. The central and the commissural strands and the leaf traces are always endarch, while the medullary strand is exarch (figs. 21, 22). The commissural strand comes off close to the central strand, and the leaf trace bifucates close to the central region. At this and the subsequent stages five vascular strands may leave the central strand approximately on the same level; namely, the eighth leaf

trace (which usually comes off divided), a root stele, the commissural strand, and the medullary strand. A section of the sporophyte at this stage is shown in fig. 6.

In the more advanced specimens studied the exact stage could no longer be determined, owing to the distortion and decay of the lower regions. The difference between these and the previous stages was shown in the earlier bifurcation of the leaf traces, which may take place as the leaf is preparing to leave the central strand.

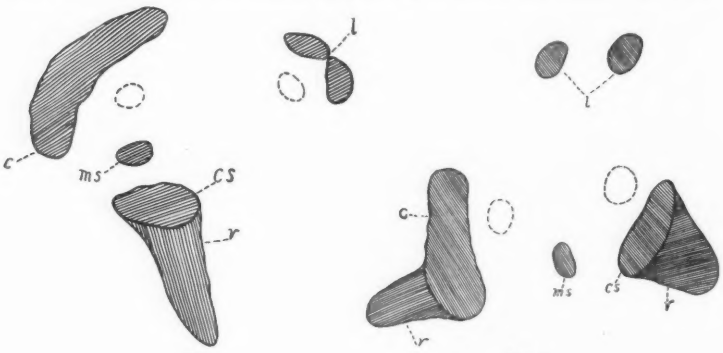


FIG. 7 FIG. 8

FIGS. 7, 8.—Fig. 7, diagram showing root attachment to commissural strand before latter has joined central strand (only xylem shown); *c*, central strand; *ms*, medullary strand; *cs*, commissural strand; *r*, root stele; *l*, dividing leaf trace; ×38; fig. 8, diagram showing two roots on same level; one joined on central strand and other on commissural strand: *c*, central strand; *ms*, medullary strand; *cs*, commissural strand; *r*, root stele; *l*, bifurcated leaf trace; ×38.

The commissural strand also appears earlier, in some cases before the leaf trace is free. The two branches of the leaf trace bifurcate while within the cortex, so that here four strands from one leaf trace enter the stipular region. Considerable variation was observed in the attachment of the commissural strand. This may run up farther before merging with the central strand, and the root which is usually attached to the central strand may be inserted on the commissural strand (fig. 7). In some cases also a root may appear higher on the central strand, bringing two roots almost on the same level (fig. 8). In such cases apparently the two roots are related to one leaf trace. In following out the series, however, it was found that

the root appearing on the central strand was related to the next leaf above, while the one attached to the commissural strand corresponds to the leaf trace above that. The appearance of roots on the commissural strand seems to be a common occurrence in the older stems.

Vascular anatomy of stipules

As has already been mentioned, no conspicuous stipules appear until the fourth leaf. At this stage the leaf trace passes through the stipules as a single strand, to which two weakly developed xylem strands are attached, which pass to the stipular lobes. In the fifth leaf these strands are better developed, and the leaf trace shows signs of forking where the stipular strands are attached. The latter remain unbranched for some distance from the leaf trace, when each gives off usually two branches to the upper part of the lobes. In the sixth leaf the trace forks where the stipular strands are given off, but anastomosing takes place immediately beyond. In the seventh leaf the point of forking of the leaf trace has moved closer to the stem, and anastomosing is delayed longer than in the previous stage. Further branching has taken place in the stipular strands. In the subsequent leaves the point of forking of the leaf trace moves closer and closer to the central region, until finally this takes place as the leaf trace is preparing to free itself from the central strand. Likewise, the point of anastomosing of the branches is moved farther and farther away from the point of attachment of the stipular strands, until the leaf trace passes into the petiole still divided. Above the tenth leaf the two branches from the forked leaf trace bifurcate, so that four strands enter the stipular region. In this region further branching and anastomosing take place. The course of the vascular bundles above the tenth leaf is represented in the reconstruction in fig. 34.

The stipular strands which pass to the lobes of the stipule bend outward and pass to within a short distance of the epidermis, where they end blindly. The lowest (or the main strand), however, is terminated by a procambial strand which originates from a group of meristematic cells on the inner side of the edge of the stipule slightly above where this merges with the cortex. This meriste-

matic region was first visible in the seventh leaf (figs. 23, 31, 34). Such a region has been reported by GWYNNE-VAUGHAN (**9**) to occur in *Kaulfussia* and *Archangiopteris*, and WEST (**13**) reports the same for two species of *Danaea*. Although not reported for the other genera of the Marattiaceae, it is probable that this region is found in them also. GWYNNE-VAUGHAN suggests that this region might represent the rudiments of the adventitious buds, and WEST reports that this does actually occur in *Danaea nodosa*. While this region may give rise to adventitious shoots, it seems probable that its principal function is to build up the fleshy stipules. In none of the specimens of *Angiopteris* studied was there any evidence of the presence of adventitious buds in this region.

Conclusions

It is evident that *Angiopteris* presents an example of striking general variation in the vascular structures from stage to stage. How far this variation is continued remains to be determined from further studies of more advanced stages than have been dealt with in this investigation. From the facts observed in the stages studied, however, it seems probable that some of these variations are continued indefinitely as the plant increases in size and age. The most conspicuous of these variations are: (1) the elimination of the endodermis, (2) the appearance of commissural and medullary strands and the increase in their importance in the structure of the central region, (3) the repeated bifurcation of the leaf traces, (4) the placing closer to the central strand the point of attachment of the commissural strand and the point of forking of the leaf trace, and (5) the variation in the place of attachment of the root steles. Much of this variation tends to the breaking up of the central strand, a fact which points to a polystelic condition so characteristic of the phylogenetically advanced types of the different groups of vascular plants.

The total absence of a cauline procambium is interesting, as it suggests that the central vascular structure in the stem of the Marattiaceae, and perhaps other closely related ferns, is mostly if not wholly of foliar origin; that is, it consists of a sympodium of leaf traces. The definite relation between roots and leaves strongly supports this theory.

Summary

1. The general internal structure of the stem of *Angiopteris evecta* consists of two main regions, the cortical and central vascular. The relatively thick cortex is traversed by leaf traces and roots. The central region consists of a vascular strand which appears crescent-shaped in cross-section and is imbedded in a central parenchyma tissue. This strand gives off root steles and leaf traces. In addition, commissural and medullary strands appear in the central region.

2. The leaf traces are given off from one edge of the central strand, and this contribution is always from the same edge in the same plant. On about the same level that the leaf trace leaves the central strand a root is attached on the outer side of the other edge. The contribution thus made to the leaf traces is made good on the opposite edge in two ways, by an increase in the vascular tissue on the edge itself, and by the addition of commissural strands.

3. The commissural strand originates from the free edge of the leaf trace after the latter is freed from the central strand, and passes to the opposite edge of the central strand and fuses with it.

4. By a continual loss of tissue from one edge and an addition on the other the central strand assumes a spiral condition. This condition is due to the spiral succession of leaves.

5. In the older stages a medullary strand appears on the inner side of the central strand opposite the upper level of a root junction. This crosses the central parenchyma tissue and fuses with the commissural strand before the latter has fused with the central strand. After fusion has taken place, the medullary strand passes upward on the inner side of the central strand, fused to the latter but retaining its identity, until the root junction has been cleared, when it frees itself and repeats its course across the central parenchyma.

6. Each leaf trace is definitely related to one root. The root which appears almost opposite a leaf trace corresponds to the next leaf above. In the early stages each leaf trace appears directly above its corresponding root; but in the later stages the leaf trace is displaced to the right or left, as the case may be, of its corresponding root, due to the spiral condition of the central strand.

7. The leaf traces at first are single, but later bifurcate in the stipular region, but anastomose beyond. The point of forking moves closer and closer to the central strand. Further forking and anastomosing of the leaf trace take place in the more advanced stages.

8. One strand from each side of the leaf trace goes to the lobes of the stipule. Branches from this strand supply the stipular lobes with vascular tissue.

9. All the stipular strands end blindly except the lowest or main strand, which is terminated by a procambial strand originating from a group of meristematic cells on the inner side of the edge of the stipule. This group of cells probably helps to build up the fleshy stipule.

10. The absence of cauline procambium and the definite relation between roots and leaves suggest that the vascular tissue of the central region is a sympodium of leaf traces, and most if not all of the central strand is of foliar origin.

The writer wishes to express his gratitude to Dr. W. J. G. LAND for the valuable collection of material furnished for this investigation and for his advice, criticism, and encouragement during the progress of the work.

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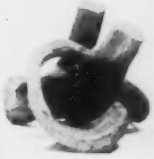
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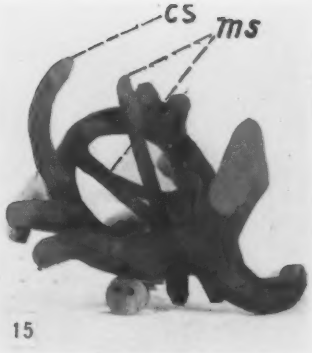

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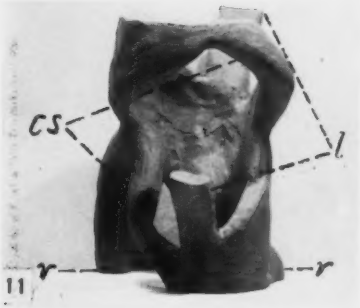

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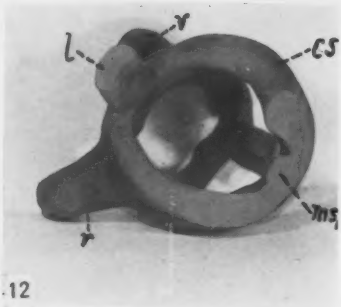

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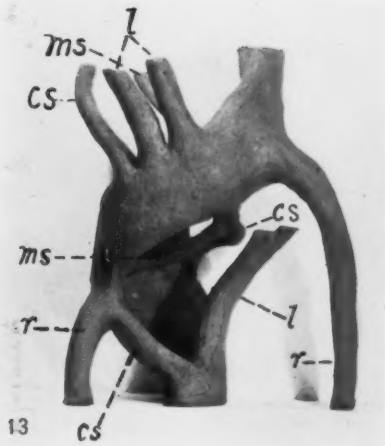

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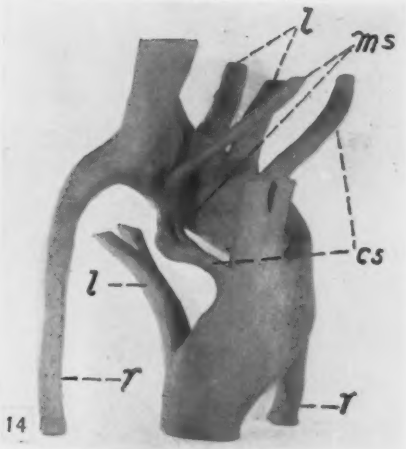

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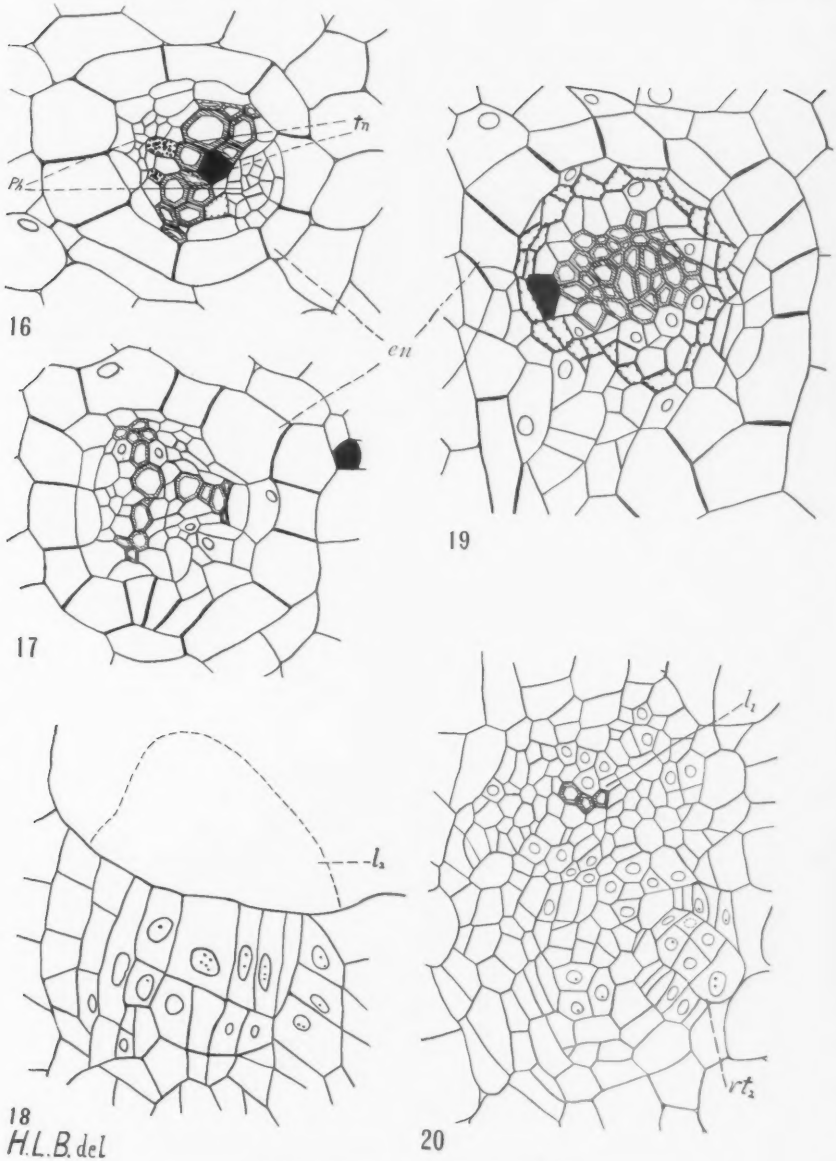
BLOMQUIST on ANGIOPTERIS



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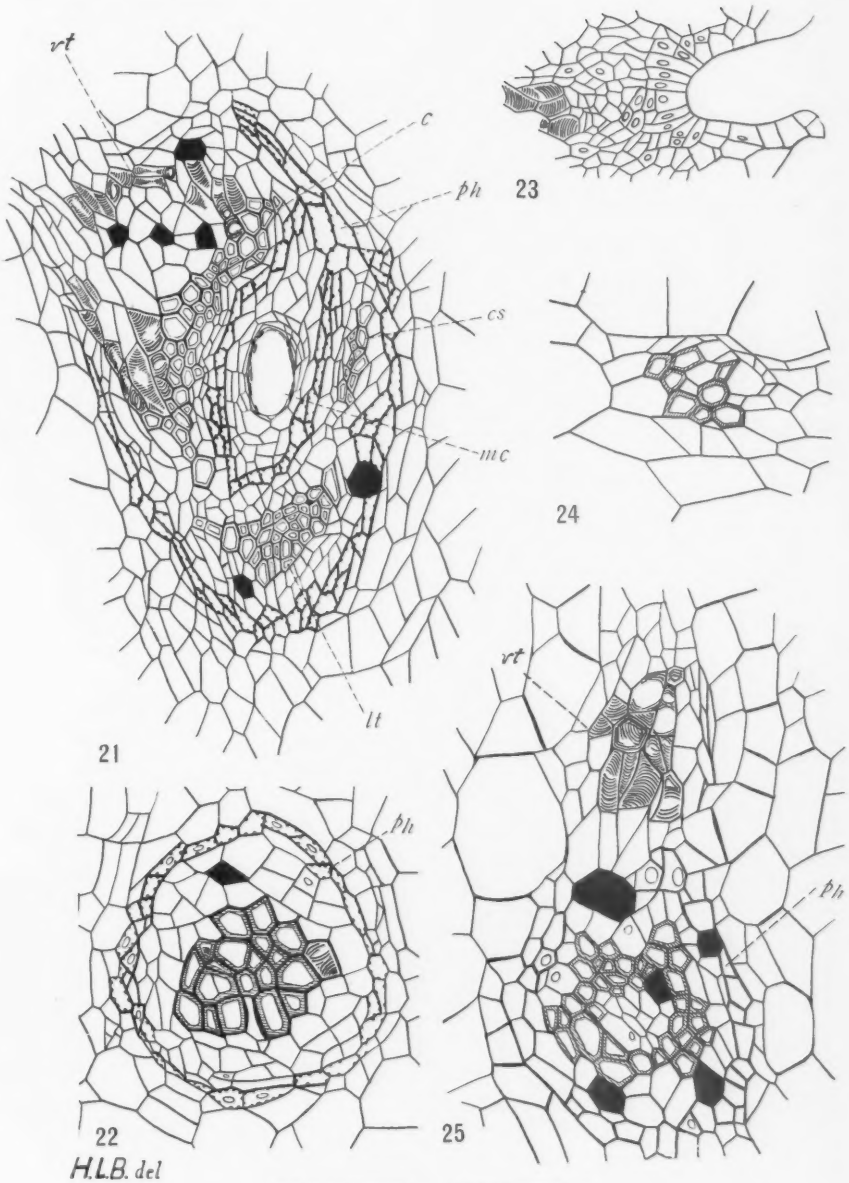

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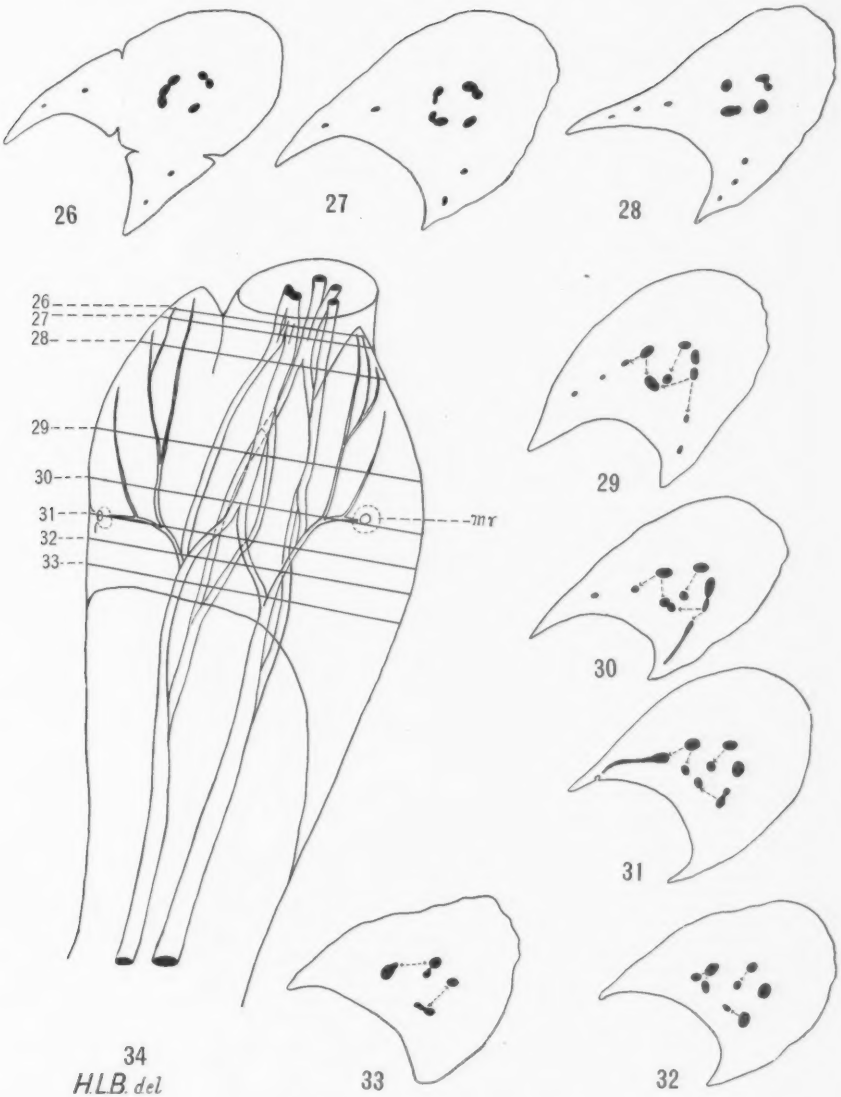

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EXPLANATION OF PLATES V–VIII

PLATE V

FIG. 9.—Model of stelar structure represented in fig. 3: r_1, r_2, r_3, first, second, and third root steles; l_1, l_2, l_3, first, second, and third leaf traces; *cs*, commissural strand; *lg*, leaf gap.

FIG. 10.—Same model viewed from above.

FIG. 11.—Model of stelar structure represented in diagram in fig. 4.

FIG. 12.—Same model viewed from above: ms_1, first medullary strand.

FIG. 13.—Model of stelar arrangement represented in fig. 5; abbreviations same as above.

FIG. 14.—Same model viewed from opposite side.

FIG. 15.—Same model viewed from above.

PLATE VI

FIG. 16.—Detail of a diarch primary root: *en*, endodermis; *ph*, phloem; *tn*, tannin cell; *pc*, pericycle; ×330.

FIG. 17.—Detail of triarch primary root; ×330.

FIG. 18.—Apical cell at stem tip: l_2, second leaf; ×330.

FIG. 19.—Detail of transition region; ×330.

FIG. 20.—Section of sporeling: rt_2, origin of second root; l_1, first leaf trace; ×330.

PLATE VII

FIG. 21.—Detail of central region on level of outgoing of fourth leaf trace: *c*, central strand; rt_5, fifth root stele; *cs*, commissural strand; *ph*, phloem; *mc*, mucilage canal; ×170.

FIG. 22.—Detail of medullary strand: *ph*, phloem; ×330.

FIG. 23.—Meristematic region terminating main stipular strand; ×180.

FIG. 24.—Detail of stipular strand; ×330.

FIG. 25.—Detail of stele above transition region showing medullated condition; rt^2, second root trace; ×330.

PLATE VIII

FIGS. 26–33.—Diagrams of sections of stipular region from advanced stage showing arrangement and inter-relation of vascular strands; ×8.

FIG. 34.—Reconstruction of sections: *mr*, meristematic region of stipule; reconstruction is slightly larger than sections to show better the courses of strands.

SYMBIOSIS IN A DECIDUOUS FOREST. I

W B. McDOUGALL

(WITH THREE FIGURES)

Introduction

In a previous paper[1] symbiosis is defined as the living together of dissimilar organisms, and the phenomena of symbiosis are classified as follows:

I. Disjunctive symbiosis
  1. Social
  2. Nutritive
    a. Antagonistic
    b. Reciprocal

II. Conjunctive symbiosis
  1. Social
  2. Nutritive
    a. Antagonistic
    b. Reciprocal

The writer has felt for some time that there is urgent need of work along the lines of these various types of symbiosis. Considerable work has been done on the relations of plants to their physical environment, but the work done on their relation to the biotic environment has not been in proportion to the importance of this phase of ecology.

Some five years ago the University of Illinois obtained possession of a sixty acre tract of forest. This land is located about five miles northeast of the University in Champaign County, Illinois, and is a remnant of a former much more extensive forest. It is known as the "University woods." Many of the primitive trees are still standing in this tract, although some cutting has been done in the past, and the forest was frequently pastured before it was acquired by the University. It may be said, therefore, to be only semi-primitive. It has been stated that there is very little if any natural forest vegetation in the state of Illinois at the present time, but the use of the word natural in such a statement is ill chosen and makes the statement inexact. It is true that there is very little if any such vegetation that has not been modified to a greater or less extent by man. Vegetation that has been influenced by man, however, is

[1] McDOUGALL, W. B., The classification of symbiotic phenomena. Plant World 21:250–256. 1918.

not necessarily "unnatural." The plants in any forest in Illinois at the present time have life problems that are just as real, just as complex, and just as interesting from an ecological point of view as were the problems of plants in the primitive forests. We should make every effort, of course, to preserve as much of the primitive vegetation as possible, but no ecologist whose location is remote from primitive vegetation need feel that there is nothing he can do, for wherever plants grow there is ecological work to be done even though the land may all be under cultivation.

The work, a part of which is recorded in the present paper, was undertaken for the purpose of making a thorough study of symbiosis in all its phases in the University woods. The present paper

RAINFALL (IN INCHES) AT URBANA; AVERAGE FOR SIXTY YEARS

Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	TOTAL
2.16	2.42	2.92	3.47	4.04	4.11	3.89	3.04	3.60	2.21	2.49	2.24	36.89

TEMPERATURES AT URBANA; AVERAGE FOR FORTY YEARS

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Aver.
Maximum	57	60	72	83	89	94	98	96	93	84	72	58	80
Minimum	− 5	− 4	12	26	36	46	52	51	39	27	14	2	25
Mean	26.6	29.2	39.8	53.0	62.9	71.9	76.4	74.4	67.2	55.3	41.5	30.4	52.4

deals primarily with social disjunctive symbiosis, and it is the hope of the writer to follow it with others treating of the other types of symbiosis.

University woods

PHYSICAL ENVIRONMENT

CLIMATE.—Table I gives a summary of weather conditions at Urbana, Illinois, during 1919. The data for this table were kindly furnished and the table compiled by the Division of Soil Physics of the Agriculture Experiment Station of the University of Illinois. There were slightly more clear days in 1919 than usual, but on the whole the table is typical of the climate of this region. The following tabulations of average monthly and annual rainfall at Urbana for a period of sixty years, and of average maximum, mini-

mum, and mean monthly temperatures for a period of forty years, were compiled from data given by MOSIER.[2] It will be noted that the rainfall is adequate and is well distributed throughout the year. The average data of the last killing frost in spring is April 26, and that of the first killing frost in autumn is October 16, giving an average growing season of 173 days.

SOIL.—The soil of the forest is yellow-gray silt loam, an upland timber soil.[3] There are no streams within the forest, but the region is drained by a tributary of the Salt Fork of the Vermillion

TABLE I

WEATHER CONDITIONS AT URBANA DURING 1919

1919	TEMPERATURE			RAINFALL IN INCHES	MEAN RELATIVE HUMIDITY	WIND		NO. OF CLEAR DAYS	NO. OF PARTLY CLOUDY DAYS	NO. OF CLOUDY DAYS
	Maximum	Minimum	Mean			Average velocity	Direction			
January	55	− 8	31.2	0.21	76.4	7.5	SW	16	6	9
February	56	11	31.5	1.92	77.0	8.4	NW	10	3	15
March	67	9	42.0	4.12	75.5	11.9	S	13	6	12
April	76	24	53.2	0.75	76.1	9.1	SW	7	6	17
May	92	38	60.8	3.29	78.6	7.4	NE	8	7	16
June	91	52	75.5	6.90	79.9	5.8	SE	10	13	7
July	96	56	81.3	2.66	59.3	6.1	SW	23	8	0
August	94	50	73.9	3.85	63.4	6.4	SW	19	9	3
September	92	42	70.5	2.47	68.1	6.5	SW	19	5	6
October	88	33	58.4	5.59	79.8	7.0	SW	11	1	19
November	63	16	39.1	3.37	78.3	8.9	SW	12	5	13
December	51	− 4	23.9	0.12	77.6	7.4	SW	9	3	19
Total	.....	.....	.....	35.25	.....	.....	.....	157	72	136
Average	76.8	26.6	53.4	2.94	74.2	7.7	SW	13.1	6.0	11.3

River. This ditch flows in an easterly direction some forty rods south of the present boundary of the forest. The forest itself is only gently rolling, but several acres in the middle eastern portion are several feet lower than the highest parts of the woods, and in the lowest part there is often standing water for a few weeks in spring.

As will be shown later, the vegetation in the lower part of the woods is quite different from that in the higher parts, and this difference seems to be almost entirely due to the difference in the

[2] MOSIER, J. G., Climate of Illinois. Univ. Ill. Agric. Exp. Sta. Bull. 208. 1918.

[3] HOPKINS, C. G., MOSIER, J. G., VAN ALSTINE, E., and GARRETT, F. W., Champaign County soils. Univ. Ill. Agric. Exp. Sta. Rep. 18. 1918.

amount of soil water. A number of measurements of soil temperature were made in both the higher and lower parts of the forest at depths of three inches and twelve inches, but temperatures taken in different parts of the forest on the same day and at the same depth were found to be almost exactly the same. Tests were made also for soil acidity in different parts of the forest, using the indicator method described by WHERRY.[4] The results of these tests showed that the soil of the forest is neutral throughout. Although no chemical analyses have been made, there is no reason for thinking that the mineral constituents of the soil are not practically the same throughout the forest.

THE PLANT COMMUNITY

METHODS.—Before the more special types of symbiosis can be studied adequately in any plant community, it is necessary to have a thorough understanding of the structure of the community and of the disjunctive social symbiosis, that is, of the gross interrelations of the component parts of the vegetation. In order to obtain this information, the entire forest was first staked out into one hundred foot quadrats. The only reason for choosing the one hundred foot quadrat instead of a unit of the metric system was that one hundred foot steel surveyor's tapes are readily obtained and are convenient to use. No attempt was made to have the quadrats exactly one hundred feet square. The tapes were fastened to the stakes by means of straps, and these were of such a length that on level land and with the tapes drawn tight the stakes would be about one hundred two feet apart. In the woods, however, with the tapes running among shrubs, between trees, over fallen logs, etc., and with the tape never very tightly drawn, the quadrats were approximately one hundred feet square. It was found that by making fractional quadrats across each end of the woods and part way along one side, to where there is a jog in the boundary line, there were then 216 full size quadrats (fig. 1).

Having subdivided the forest into quadrats, two methods were used for locating and mapping the components of the vegetation. The first method was used only for the trees and shrubs. Two

[4] WHERRY, E. T., Soil acidity and a field method for its measurement. Ecology 1:160–173. 1920.

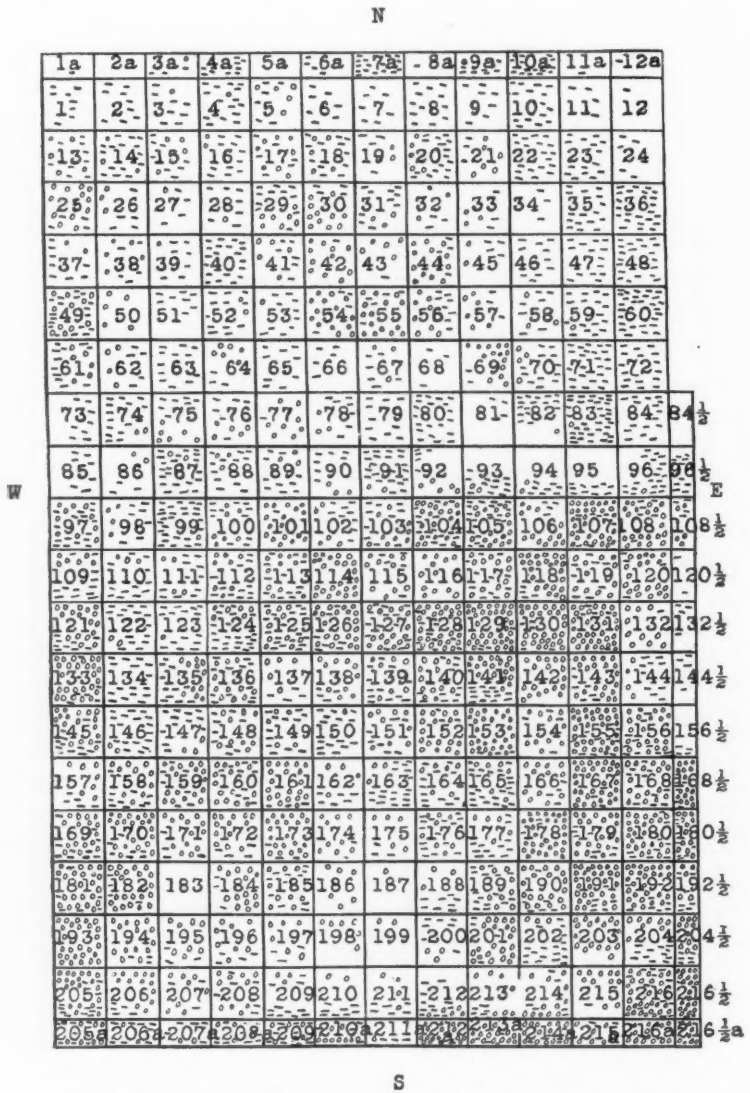

FIG. 1.—Quadrat map of University woods showing distribution of the two dominant trees: o = *Acer saccharum*, — = *Ulmus americana*.

rows of quadrats were surveyed at the same time. A hundred foot tape was stretched between the first two quadrats of the first two rows. Then while walking through these quadrats in the directions indicated in fig. 2, counts were made of all species of trees and shrubs present. The frequency of the occurrence of each species of shrub was indicated by the numerals 1, 2, 3, and 4; no. 1 indicating only one to three specimens in the quadrat, no. 4 indicating great abundance, and nos. 2 and 3 representing intermediate degrees of frequency. In the case of trees the actual

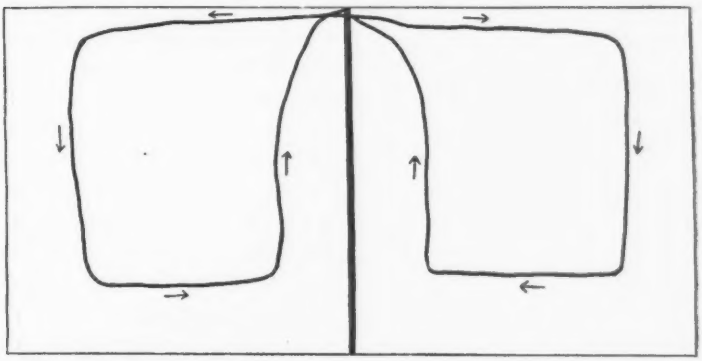

FIG. 2.—Two one-hundred-foot quadrats illustrating method used in making counts of trees and shrubs in University woods: heavy line between quadrats indicates position of steel tape; arrows show direction taken by surveyor in making counts.

number of individuals of each species was noted. After counts in these two quadrats had been completed, the tape was taken up and stretched between the next two quadrats and counts made there in a similar way, the procedure being repeated until the entire forest had been surveyed. Having secured these data, a distribution map was made for each species (fig. 1). No attempt was made to locate the individuals within the quadrat, but only to place them in the quadrats in which they occur. Each individual is shown on the maps, therefore, within one hundred feet or less of its actual position in the forest.

For the herbaceous vegetation a somewhat different method was used. Only one row of quadrats was surveyed at a time and no

tape was used. Instead, a pocket compass was employed for keeping on a line running through the middle of the row of quadrats, and each quadrat was surveyed by walking through it in the manner indicated in fig. 3. The middle of each quadrat after the first was found by pacing thirty-three paces from the middle of the preceding quadrat with the compass as a guide to the direction. The place was then marked by sticking into the ground a stick about four feet long which was sharpened to a point at the lower end and whittled off at the upper end so as to give a whitish surface which could be seen for some distance. This stick was carried from quadrat to quadrat as the work progressed. The various species of herbaceous plants present in each quadrat were recorded as nos. 1, 2, 3, or 4; no. 1 signifying rare, no. 2 frequent, no. 3 common, and no. 4 abundant. A map was then made for each species, showing its distribution and relative frequency of occurrence throughout the forest. In surveying for herbaceous plants, only those plants that were in bloom or were otherwise easily recognized were listed; and in order to make the lists complete the survey was repeated once each month during the growing season.

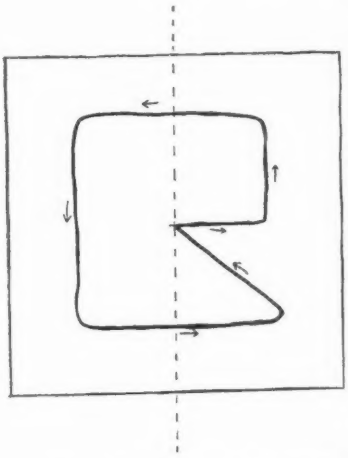

FIG. 3.—A one-hundred-foot quadrat illustrating method used in estimating frequency of herbaceous plant species in University woods: broken line with arrows indicates direction taken in locating center of quadrat; solid line with arrows shows directions taken in making survey.

RESULTS.—By this method a fairly complete list of the plants growing in the woods was obtained, together with the relative numbers of individuals and the locations of each species. No attempt has been made so far to include the bryophytes and the microfungi and algae in the survey, although it is hoped that this may be done later. The bryophytes are nowhere abundant, and

probably are of relatively little importance from the viewpoint of symbiosis or other ecological relations. This is obviously even more true of the algae in such a habitat as this. The microfungi, however, will have to be considered in some detail later in a discussion of their symbiotic relations with other plants. Aside from these three groups, the lists of species are believed to be fairly complete, except that there are probably a few late fall-blooming plants, mostly composites, that have been missed owing to the fact that the last survey of the season was interfered with by duties in connection with the opening of the University and the beginning of classwork for the year.

The lists of species which have been compiled include 31 trees, 12 shrubs, 6 lianas, 134 herbs, 5 ferns, and 83 higher fungi. These figures show that while there is by no means a paucity of species, yet for a sixty acre deciduous forest the flora cannot be said to be an especially rich one. It is not unlikely that the years preceding the acquirement of this property by the University, when it was frequently pastured and the public was allowed to dig up plants by the roots and carry them away at will, had their effect in reducing the number of species.

Acer saccharum and *Ulmus americana* are the dominant trees. These two are present in nearly equal numbers, the count showing 1987 maple and 2073 elm individuals. No other species are nearly so abundant as these two. The nearest competitor is *Fraxinus americana* with 537 individuals, then follow *F. quadrangulata* with 336, *Tilia americana* with 321, and *Carpinus caroliniana* with 303 representatives. All other species are represented by less than 300 individuals. These data show at once that the forest is typically hydrarch mesophytic and relatively mature, *Acer saccharum* being the most typical climax species of the region. While these species are all found more or less throughout the forest, the maple is much more dominant in the southern half and the elm in the northern half. In fact three-fourths of the hard maple and two-thirds of *Fraxinus quadrangulata* are in the southern half of the woods, while three-fifths of the elm, three-fifths of *F. americana*, and two-thirds of the blue beech (*Carpinus*) are in the northern half. It is the higher parts of the woods that are dominated by the maple,

while the lower parts are dominated by elm. In the area between quadrats 59 and 96 and vicinity (fig. 1), which is the lowest part of the woods, there are almost no trees except elms. I shall frequently refer to that part of the woods that is dominated by maple as the maple consociation, and that dominated by elm as the elm consociation.

The great majority of the shrubs are of two species, *Asimina triloba* and *Benzoin melissaefolium*. Both of these are very abundant, and are distributed throughout the forest except in the highest and lowest parts. No other shrubs are abundant except the species of *Crataegus*, which occur mostly along the border.

The subdominants of the herbaceous layer vary with the season as well as with the location within the forest. In the prevernal season *Claytonia virginica*, *Isopyrum biternatum*, and *Collinsia verna* often form extensive and conspicuous societies in the maple consociation. The two species of *Dicentra*, *D. cucullaria* and *D. canadense*, occur together as subdominants in a rather extensive prevernal society. *Phlox divaricata* and *Geranium maculatum* form less extensive but not less well marked societies. *Asarum canadense* is everywhere abundant, but because of its growth habit is not conspicuous. *Viola sororia* is abundant, but is scattered too much to be considered a subdominant. In the elm consociation *Floerkia proserpinacoides* forms an extensive and extremely dense society. Like the elm this plant is present throughout the forest, but is always thickest where the elms are dominant.

During the vernal season *Hydrophyllum appendiculatum* forms an extensive society in the maple consociation. Later *H. canadense* is a subdominant over less extensive but more closely occupied areas. *Cystopteris fragilis* is abundant over considerable areas, and locally is present in such numbers as to become a subdominant. *Podophyllum* is also common, but as usual forms local colonies rather than extensive societies. All of these societies extend into the elm consociation, but there is no subdominant that is characteristic of the elm consociation during this season.

The aestival season is characterized by the subdominance of *Laportea canadensis* over great areas. Like the dominant shrubs *Asimina* and *Benzoin*, the wood nettle is abundant throughout

the woods except in the highest and lowest parts. At the same time *Impatiens biflora* is subdominant in the lower parts of the elm consociation, while *I. palida* is only a little less prominent, and keeps largely to the somewhat higher parts of the same consociation.

During the serotinal season several composites are conspicuous, but perhaps the most characteristic plant is *Campanula americana*, which occurs nearly everywhere in the woods, but usually not abundantly enough to become subdominant. Finally, during the autumnal season species of *Aster* and *Eupatorium urticaefolium* are the principal subdominants.

Social disjunctive symbiosis

This is the type of symbiosis in which the organisms concerned are not in actual contact, at least not all of the time, and in which there is no direct food relation. It includes, therefore, all of the ordinary interrelations of dominant, subdominant, and secondary species in a plant community. These interrelations in deciduous forests have been studied and described by numerous authors. It will suffice here, therefore, merely to mention the salient features of the subject, and to point out their relative importance in the community under consideration. The dominant plants of a community, which in a forest are trees, are those which largely control the environment and so determine what other species may grow in the community. They have very important symbiotic relations, therefore, with all other members of the community through their direct or indirect control of light, space relations, water supply, and to a certain extent available food materials. From this point of view it is of interest to compare a plant community with a human community. In a human community man is the dominant species. As the dominant species he controls the environment to such an extent as to determine what other species may live in the community. Some of the other species usually found in a human community are the horse, dog, cat, mouse, fly, etc. Some of these are not present because man wants them to be, but because man is present and is controlling the environment in such a way as to make it possible for the other species to live in the community. These facts are just as true of the plant community. The presence of

some of the species is distinctly advantageous to the dominant plants, while that of others is just as distinctly disadvantageous, as for example the parasitic fungi, but they are all present because the dominant plants have made it possible by their control of the environment. In the human community we find a well marked division of labor among the individuals of the dominant species; some are engaged in supplying food, others in supplying clothes or fuel, others in administering the law, etc. In the plant community we find a somewhat comparable division of labor among the various species of the community, but not among the individuals of the dominant species. The function in the community of all members of the same species is the same, but some species have the function of manufacturing food, some for supplying a ground cover to check evaporation from the soil, some to act as scavengers in getting rid of dead bodies, etc. Another important difference between the human community and the plant community should be kept in mind. In the human community there are ordinarily more or less definitely organized activities carried on for the good of the community as a whole. On the other hand, in the plant community there is no altruism. It is a case of every plant for itself. The activities of certain species do result advantageously for the community as a whole, but this is due to chance circumstances, and the activities of course would be carried on just as vigorously if they were resulting in harm to the community. This fundamental difference between the two communities, however, is the natural result of the presence of consciousness in the human species and the lack of it in plants, and as soon as we leave that fact out of consideration the two types of communities become strikingly similar.

The individuals of any species, whether a dominant or a secondary species in a plant community such as the one we are considering, all make similar demands upon the environment. For this reason their relations seldom result in any benefits, but on the other hand there is constant competition between them for space, food, and often for other environmental factors such as light or shade. This is often just as true of individuals of different species which make such similar demands upon the environment as to merit being called ecological equivalents. Species which are ecologically

very different, on the other hand, often are incidentally very serviceable to one another. The trees, for example, furnish the shade necessary for some of the herbaceous plants and fungi, while the herbaceous plants furnish a living soil cover which prevents undue loss of the soil water which is needed in great quantities by the trees. The trees, likewise, as well as the shrubs, especially those near the border of the woods, serve as a windbrake which protects many smaller plants from the dangers of too high transpiration rates.

Of very great importance from the viewpoint of social disjunctive symbiosis is the phenomenon of leaf fall. The primary reason for leaf fall, of course, is the reduction of the transpiration surface during the season when absorption is difficult or impossible, and the primary cause is desiccation, but the effect of this habit on other members of the community is perhaps as important as its significance to the deciduous plants themselves. The fallen leaves form an efficient cover throughout the winter season, thus greatly reducing evaporation from herbaceous perennial plants as well as from the surface of the soil. The place of the fallen stems of herbaceous perennials in social disjunctive symbiosis is similar to that of the fallen leaves, as, likewise, is that of the dead bodies of annual plants.

Closely connected with leaf fall are the activities that are concerned with the decay of the fallen leaves. These are due mostly to bacteria and fungi. The bacteria and fungi are regular members of the community, and are living in social disjunctive symbiosis with the higher plants. They are able to live in the community only as a result of the presence of the higher plants, and they render a distinct service to the community by preventing the accumulation of dead bodies.

Although the phenomena just cited all result in benefit to certain members of the community, it must be understood that symbiosis does not necessarily imply any benefit to the symbionts, mutual or otherwise. In antagonistic symbiosis there is often more harm than benefit for at least some of the symbionts, while in social symbiosis there may be a mere tolerance of presence with neither harm nor benefit resulting to any appreciable extent. Thus many of the species in a community such as the one under

consideration are able to live together largely because the main parts of their absorbing systems are placed at different levels in the soil.[5] For example, *Circaea lutetiana* has its rhizomes only about one inch beneath the surface of the soil; the rhizomes of *Asarum canadense*, *Sanguinaria canadensis*, and *Thalictrum dioicum* are about two inches deep; those of *Podophyllum peltatum* and *Sanicula gregaria* average about two and one-half inches deep; the bulbs of *Allium canadense* are placed about three inches, and the corms of *Arisaema triphyllum* about five inches below the surface of the soil. The rhizomes of *Polygonatum commutatum* are produced about three inches down, and are later pulled down by root contractions to a depth of five or six inches. Along with these, of course, are the trees and shrubs which have absorbing organs at all depths to a distance of several feet. Still another factor which tends to make it possible for large numbers of species to live together in a forest community is the fact that different species carry on their more important activities at different times of the year, and so do not interfere with each other as much as they otherwise would. It is this that makes it possible to distinguish prevernal, vernal, aestival, serotinal, and autumnal seasons, each characterized by the prominence of different groups of species.

It is not necessary, however, to carry the discussion further along this line. It is recognized that there is nothing new in the preceding discussion except the point of view. In other words, the kinds of interrelations here pointed out are well known to most botanists, and for that reason it was considered unnecessary to go into much detail; but these interrelations have not usually been considered as cases of symbiosis. The reason for dwelling upon them here, therefore, has been to emphasize the fact that they are instances of the living together of dissimilar organisms, and so properly belong in a discussion of symbiosis. This proper point of view is preparation for further discussion of the other types of symbiosis, some of which are not so well known.

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[5] SHERFF, E. E., Vegetation of Skokie Marsh. Ill. Sta. Lab. Nat. Hist. Bull. 9:575–614. 1913.

EFFECT OF TEMPERATURE ON GERMINATION OF AMARANTHUS RETROFLEXUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 291

CLYTEE R. EVANS

(WITH FOUR FIGURES)

Introduction

The literature having a direct bearing on the effect of temperature on germination may be divided into two groups: one deals with growth in relation to temperature, and the other with delay in germination of seeds in general and of *Amaranthus retroflexus* in particular. In the first group the articles by SMITH (10), LEHENBAUER (7), LEITCH (8), BALLS (1), and KANITZ (6) on relation of growth to temperature are of interest. SMITH found that temperature, possibly internal temperature of the growing parts, may be a limiting factor to growth in *Furchraea* and *Agave*. LEHENBAUER, in his work on rate of growth of maize seedlings, found that the Van't Hoff law applies only at medium temperatures; at 31° C. the initial rate is not maintained, there being a falling off with time. He further found that the coefficients for 10° C. rise in temperature are greater at lower ranges of temperature (6.56 at 12°–22° C.), and less (0.06 at 33°–43° C.) at higher ones. He states that the optimum changes with length of exposure, and that there are not two optima, as stated by KOEPPEN. Miss LEITCH, in work with rate of growth of seedlings of *Pisum sativum*, found that the Van't Hoff law applies only from 10° to 28° or 30° C.; that there is the same type of gradation in the coefficients relating rate of growth to temperature that LEHENBAUER found; and that above 29° C. the relation of growth to temperature can no longer be expressed as a curve, so that a different curve must be constructed to express the rate of growth in successive time intervals. She defined the optimum temperature as the highest one at which the time factor does not enter. BALLS offers an explanation of the time factor, and says

that the Van't Hoff law applies approximately up to 30° C., then the growth rate acceleration decreases to a point which he proposes to call the "stopping point," a point below the lethal temperature.

KANITZ has written a monograph on the effect of temperature of life processes in which he cites over three hundred pieces of literature distributed among several fields. The following is based on DENNY'S review of this monograph. He derives formulae from those of BERTHELOT, ARRHENIUS, VON ESSEN, and VAN'T HOFF, by means of which the value of Q_{10}, the coefficient for 10° C. rise in temperature, can be calculated from experimental data at any two temperatures. These formulae are $Q_{10}=\left(\frac{k_2}{k_1}\right)^{\frac{10}{t_2-t_1}}$ and $Q_{10}=10^{\frac{10(\log k_2-\log k_1)}{t_2-t_1}}$, in which k_2 = rate of process at temperature t_2, and k_1 = rate of process at temperature t_1. He found that when he calculated results at short temperature intervals instead of long ones, Q_{10} is often not a constant at all intervals, but falls at high temperatures. He also states that many processes in plants and animals exhibit a temperature coefficient the same as the Van't Hoff one within certain temperature limits, and cites in this connection such plant processes as CO_2 assimilation between 0° and 37° C. (MATTHAEI); respiration of seedlings between 0° and 35° C. (KUIJPER); water intake of barley grains between 3.8° and 34.6° C. (BROWN and WORLEY); permeability of plant cells and tissues between 0° and 30° C. (RYSSELBERGHE); etc. Some of these processes show higher values of Q_{10} at lower temperatures, or temperatures near the minimum of the process.

BEAL (**2**) has found that seeds of *Amaranthus retroflexus* are long lived; that they are still viable after burial in the ground for thirty years. Delays in germination of seeds are due (putting aside the stimulus idea held by some workers) either to embryo characters such as immaturity of embryo, or need of fundamental chemical changes in the embryo preceding germination in a seemingly otherwise mature embryo (**5**), or to coat effects acting jointly with embryo characters. The embryo in the latter case is not dormant when naked and exposed to ordinary germinative conditions.

These coat effects may be of several kinds, namely, the almost complete exclusion of water from the embryo, as in some Leguminosae, the cutting down of oxygen supply below the minimum required for germination (9), or the high elasticity or breaking strength of the coats as compared with the force of the expanding embryo (3, 4).

CROCKER (3) and CROCKER and DAVIS (4) state that this last named coat effect is the chief cause of dormancy in seeds of *Amaranthus retroflexus*. This dormancy gradually disappears in dry storage, as is shown by a continual lowering of the minimum temperature for germination. On the other hand, wild oats, "rain barley," and a South American grass, *Chloris ciliata*, have a low maximum when not after-ripened, which rises as after-ripening progresses. Even in seeds of *Amaranthus retroflexus* which have been stored for a long time, incompleteness of after-ripening is indicated by the considerable lingering effects of the coats. Fully after-ripened seeds have their minimum for germination lowered by removal of coat restrictions. In fresh *A. retroflexus* seeds with coats treated, the minimum temperature for germination is the same as in dried seeds with coats treated. Vigor of the embryo of *A. retroflexus*, ability to respond in germinative conditions, and the rate of growth of the naked embryo under any given conditions is not affected noticeably by after-ripening. In this seed after-ripening seemingly is not a matter of after-ripening of the embryo, for embryos of fresh seed are of maximum vigor if coat effects are removed. That the breaking strength of these coats is lowered by a rise in temperature is shown by the fact that ripe seeds gathered from green plants will not germinate at temperatures lower than 40° C., but will germinate slightly at that temperature. *A. retroflexus* seeds are slightly inhibited by light at all temperatures, according to the unpublished experiments of CROCKER and DAVIS. This holds for seeds in which the coats are treated as well. Knowledge of these conditions was requisite to handling the material intelligently in finding the series of coefficients relating rate of germination of *A. retroflexus* to temperature changes.

Material and method

Three lots of seeds were used: one gathered in late summer or early fall, 1915, at Pullman, Washington; and the other two at Gary, Indiana, one lot in the late summer or early fall of 1914, and the other a year later. The experiments extended from March 15 to August 1, 1916. Thus after-ripening had proceeded for several months in two lots, and for one year and several months in the third lot. In some of the experiments, remaining coat effects were further eliminated by grinding some of the seeds with sand for four, six, or nine weeks, or treating them with concentrated H_2SO_4 for two or three minutes and then washing them with running tap water for two minutes. The optimum length of time for treatment with H_2SO_4 was determined by experiment to be two minutes for Indiana seeds, and three minutes for Washington seeds. Along with these treated seeds were run seeds with coats not treated. These seeds, treated and untreated, were then put in lots of one hundred in Petri dishes lined with moist absorbent cotton. The Petri dishes were placed in a cardboard box lined with opaque black paper, and the box then put in a refrigerator for forty-eight hours, where the temperature was below the minimum necessary for germination, to allow time for the seeds to soak before they were tested for temperature effects on rate of germination. At the end of this time the box containing the Petri dishes was kept at the desired constant temperature. The temperatures tested ranged from 9° to 42° C. Temperatures below room temperature were obtained in baths cooled with running water, and at room temperature and above, in a constant temperature incubator in which the variation was less than one degree. At one time only did the temperature vary more than this, and then it was in the water-cooled bath; this variation is indicated on the curves 1, 2, and 3, and in all the tables thus, (8°–10° C.).

The percentage germination at various intervals of time for each temperature tried was noted for all seeds in the box. The results were plotted as curves; one set of curves for each type of seeds, that is, one set of curves for Indiana seeds collected in 1915 and treated with H_2SO_4 for two minutes, another set for Indiana seeds collected in 1915 and ground with sand nine weeks, another

set for Indiana seeds collected in 1915 and untreated, another for Washington seeds untreated, etc. Eleven sets of curves were thus plotted, three of which are included in this paper as fairly typical ones. Not fewer than two hundred seeds of each type were germinated at each temperature, one hundred each in a separate Petri dish, so that all determinations were made at least in duplicate. In some instances four hundred seeds of each type were used, making quadruplicate determinations.

From these curves, three sets of which are given as typical, the length of time required for certain percentage germination at two different temperatures was read, and these readings and temperatures used in the KANITZ formula: $Q_{10}=\left(\frac{k_2}{k_1}\right)^{\frac{10}{t_2-t_1}}$, in which k_2 = rate of germination at temperature t_2, and k_1 = rate of germination at temperature t_1. The rate of germination in each case of course was the percentage germination divided by the number of hours required for this to take place. The results of these computations are given in tables I to XI.

Again similar experiments were run and attempts made to secure lots of seeds at the same percentage germination with hypocotyls the same length when the seeds were subjected to different temperatures. These readings were used exactly as were the ones from the curves previously mentioned, and as they gave the same type of coefficients, for the sake of brevity are omitted here.

TABLE I

INDIANA SEEDS, COLLECTED 1915, UNTREATED

Temperature interval (° C.)	Q_{10} at certain percentage germination*									
	12	14	20	28	30	40	50	60	70	80
11.9–16.3	11.69	12.14	11.91	17.6	.....	.....	.....	.....	.....	.....
16.3–25.3	.....	2.95	3.05	2.94	2.87	3.68	4.01	.....	.....	.....
25.3–36.4	.....	1.71	1.76	1.83	1.85	1.89	2.01	2.06	2.04	2.21
36.4–42	.....	1.14	1.13	Less than 1	Less than 1	Less than 1	1.14	1.28	1.49	1.37
16.3–36.4	.....	2.19	2.25	.....	2.25	2.55	2.74	.....	.....	.....
23.9–36.4	.....	1.68	1.72	2.11	2.02	2.29	2.41	2.96	2.94	3.53

* No germination at 8°–10° in 168 hours.

TABLE II

INDIANA SEEDS, COLLECTED 1915, GROUND WITH SAND NINE WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*									
	12	14	20	30	36	40	50	60	70	80
(8 to 10)–16.3....	10.01	8.34	.....	.....	.....	.....	.....	.....	.....	.....
16.3–21.6.....	4.43	4.33	.....	.....	.....	.....	.....	.....	.....	.....
21.6–29.8.....	1.20	.....	1.31	1.30	.....	1.30	1.32	1.36	1.45	1.44
29.8–42.....	1.677	1.641	.....	.....	.....	.....	.....	.....	.....	.....
16.3–23.9.....	.....	.....	.....	.....	3.65	3.55	3.42	3.27	3.13	2.88
23.9–42.....	.....	.....	.....	.....	1.27	1.29	1.27	1.29	1.33	1.32

* 15 per cent germination at 8°–10° in 156 hours.

TABLE III

INDIANA SEEDS, COLLECTED 1915, TREATED WITH H_2SO_4 TWO MINUTES

Temperature interval (° C.)	Q_{10} at certain percentage germination							
	20	30	40	50	60	70	75	80
(8 to 10)–11.9...	1.778	2.118	5.91	.....	.....	.....	.....	.....
11.9 –14.45....	7.48	6.16	5.22	6.97	10.44	12.36	28.73	.....
14.45–23.4.....	3.69	3.81	3.82	3.83	3.66	3.70	3.74	4.27
23.4 –36.4.....	1.999	1.89	1.95	1.89	1.96	1.87	1.95	2.07
36.4 –42.....	1.93	1.74	1.45	1.32	1.23	1.27	1.25	1.161
14.45–28.9.....	2.81	2.92	2.73	2.39	2.18	1.76	.....	.....
21.6 –28.9.....	1.53	1.89	1.88	1.74	1.57	1.497	.....	1.903
14.45–36.4.....	2.03	2.53	2.57	2.53	2.53	2.49	.....	.....

TABLE IV

INDIANA SEEDS, COLLECTED 1915, GROUND WITH SAND FOUR WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination									
	4	8	10	20	30	40	50	64	70	80
(8 to 10)–23.9.....	4.82	4.74	.....	.....	.....	.....	.....	.....	.....	.....
16.3–23.9.....	.....	.....	.....	.....	.....	.....	.....	2.33	2.50	2.91
23.9–36.4.....	1.61	1.60	1.90	1.87	1.91	1.93	1.86	1.88	1.84	1.94
36.4–42.....	.....	.....	.....	1.28	1.24	1.25	1.13	1.02	1.10	1.09
(8 to 10)–21.6.....	5.75	6.05	.....	.....	.....	.....	.....	.....	.....	.....
23.9–29.8.....	.....	.....	2.79	1.93	1.91	1.83	1.75	.....	1.22	1.65

TABLE V

WASHINGTON SEEDS, COLLECTED 1915, UNTREATED

Temperature interval (° C.)	Q_{10} at certain percentage germination*				
	2	8	10	12	15
11.9 –14.45	8.173	10.32	11.75	.....	5.1
14.45–25.7	.....	2.40	2.37	.....	2.89
25.7 –36	.....	1.40	1.33	.....	1.38
36 –42	0.1928	.....	.....	.....	.....
23.9 –36	.....	1.15	1.11	.....	1.17
14.45–36	2.57	1.86	1.79	1.83	.....

* No germination at 8°–10° in 168 hours.

TABLE VI

WASHINGTON SEEDS, COLLECTED 1915, TREATED WITH H_2SO_4 THREE MINUTES

Temperature interval (° C.)	Q_{10} at certain percentage germination*					
	10	20	30	40	50	58
11.9 –14.45	5.77	5.77	5.66	5.15	1.86	1.0
14.45–23.4	3.82	3.40	3.30	4.05	4.52	5.17
23.4 –36.4	1.95	1.84	1.70	1.57	1.52	1.47
36.4 –42	2.36	1.37	1.21	1.21	1.13	1.08
21.6 –29.8	1.72	2.26	2.20	2.03	.....	.....
14.45–36.4	2.55	2.36	2.21	2.14	2.33	.....
14.45–29.8	2.56	2.32	1.98	1.60	.....	.....

* Only 2 per cent germination at 8°–10° in 84 hours.

TABLE VII

WASHINGTON SEEDS, COLLECTED 1915, GROUND WITH SAND FOUR WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*						
	16	20	30	36	40	50	54
11.9 –14.45	9.07	8.84	8.09	6.74	.....	.....	.....
14.45–25.7	.....	.....	.....	3.05	.....	.....	.....
25.7 –37.6	.....	.....	.....	1.18	1.59	1.75	1.91
14.45–37.6	2.16	2.10	2.03	2.026	.....	.....	.....

* 8 per cent germination 8°–10° in 156 hours.

TABLE VIII

WASHINGTON SEEDS, COLLECTED 1915, GROUND WITH SAND SIX WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*						
	10	20	30	40	50	60	70
16.3–23.4	2.28	2.10	2.05	.....	.....	.....	.....
23.4–42	1.45	1.36	1.24	.....	.....	.....	.....
16.3–21.6	2.62	2.80	2.58	2.67	2.34	2.29	.....
21.6–29.8	2.38	2.07	2.25	2.21	2.37	2.60	.....
16.3–29.8	2.48	2.32	2.37	2.38	2.35	2.47	2.65
29.8–42	.....	0.715	0.5799	.....	.....	.....	.....

* No germination at 8°–10° after 168 hours, and temperatures between this and 16.3° not tried.

TABLE IX

WASHINGTON SEEDS, COLLECTED 1915, GROUND WITH SAND NINE WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*				
	10	15	20	22	30
16.3–21.6	2.29	.....	2.33	.....	2.79
16.3–23.9	1.99	1.74	1.48	1.44	.....
23.9–42	.....	1.65	1.79	1.85	.....
16.3–29.8	2.53	.....	2.54	.....	2.95
21.6–29.8	2.68	.....	2.64	.....	3.06

* No germination at 8°–10° after 168 hours, and temperatures between this and 16.3° not tried.

TABLE X

INDIANA SEEDS, COLLECTED 1914, UNTREATED

Temperature interval (° C.)	Q_{10} at certain percentage germination						
	12.5	15	20	30	40	50	60
11.9 –14.45	12.82	9.60	13.15	16.85	.....	.....	.....
14.45–23.9	3.92	4.37	4.56	4.62	4.47	.....	.....
23.9 –42	.....	.....	.....	1.54	1.55	1.55	1.42
14.45–42	.....	.....	.....	2.42	2.23	.....	.....

TABLE XI

INDIANA SEEDS, COLLECTED 1914, GROUND WITH SAND TWO WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*						
	22	30	40	50	60	70	80
11.9 –14.45.....	8.75	8.88	8.30	8.001	6.65	11.42	.....
14.45–23.4.....	3.58	3.58	3.84	4.19	4.54	4.91	5.40
23.4 –36.4.....	1.92	1.91	1.89	1.89	1.92	1.88	1.68
36.4 –42.....	.....	1.24	1.22	1.18	1.10	1.06	1.35
14.45–29.8.....	2.91	3.03	3.24	3.41	3.72	3.94	4.35
23.4 –29.8.....	1.93	1.97	2.14	2.09	2.12	.....	.....
14.45–36.4.....	2.44	2.48	2.53	2.61	2.75	2.79	2.72

* 5 per cent germination at 8°–10° in 168 hours.

Discussion

In the effort to find a representative end point, many different ones were tried, with the result that almost any one proved satisfactory. These end points are Q_{10} at different percentage germination. A study of the tables shows that almost any percentage germination up to the total at the temperature might well serve for this representative end point, as in practically every series the coefficient for 10° C. rise in temperature is more than three when computed at temperature intervals near the minimum temperature for germination, and gradually decreases to about one or even less than one near the maximum. At some range between these, of course, Q_{10} falls between two and three. It is interesting that by computing through a long temperature interval Q_{10} may be found to be between two and three, when computations over shorter intervals of this longer one show Q_{10} not a constant but a variable. Take, for instance, from table III Q_{10} at the range of temperature 14.45° –36.4° = 2+, while Q_{10} at 14.45°–23.4° = 3.6 to 4+, 23.4°–36.4° = 1.8 to 2.0. Here Q_{10}, computed through a long interval where its values range from 4 to 1.8, gives an average value of 2+. This is the sort of thing to which KANITZ called attention. The series of coefficients computed through short temperature intervals agrees in general type with those given by LEITCH for effect of temperature on rate of growth of seedlings of *Pisum sativum*, with those given by LEHENBAUER for rate of growth of corn seedlings in

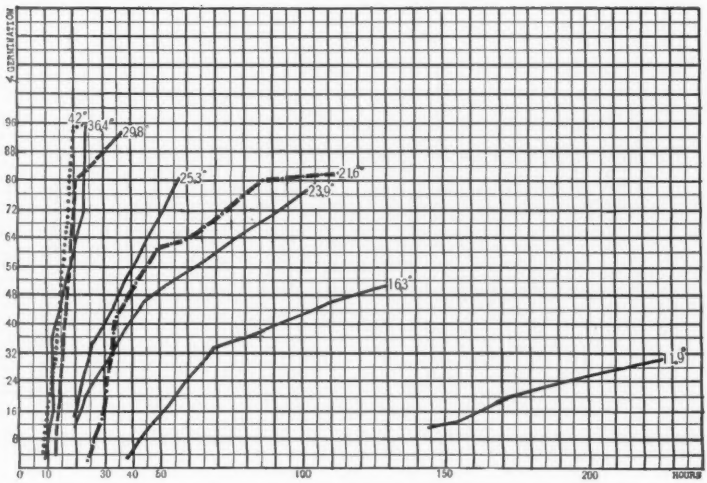

FIG. 1.—Rate of germination at certain temperatures (° C.) of seeds of *Amaranthus retroflexus*, collected at Gary, Indiana, in 1915, and untreated.

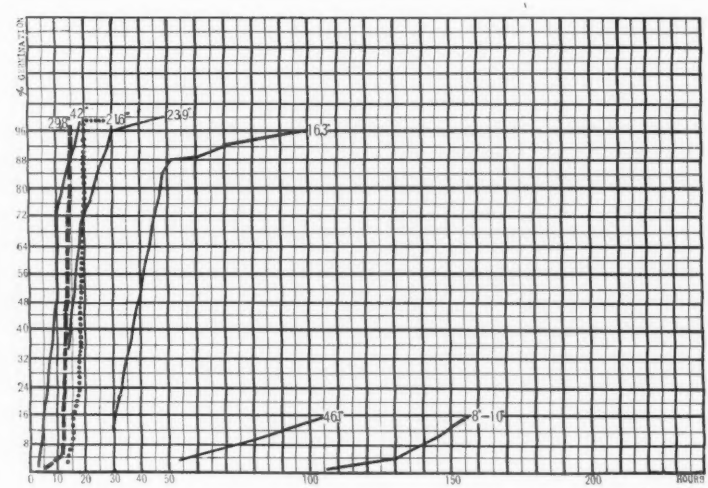

FIG. 2.—Rate of germination at certain temperatures (° C.) of seeds of *Amaranthus retroflexus* collected at Gary, Indiana, in 1915, and ground with sand nine weeks before being set to germinate.

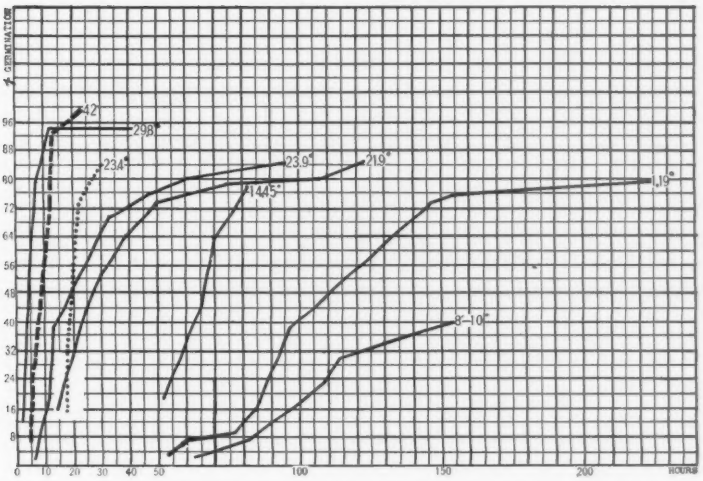

FIG. 3.—Rate of germination at certain temperatures (° C.) of seeds of *Amaranthus retroflexus* collected at Gary, Indiana, in 1915, and treated with concentrated H_2SO_4 two minutes before being set to germinate.

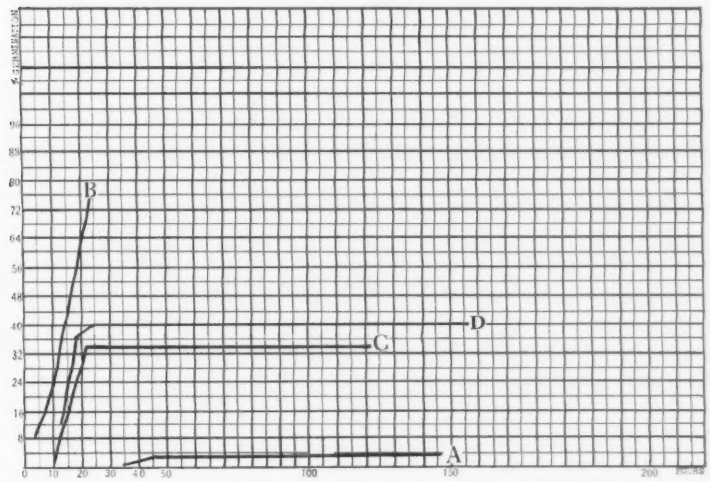

FIG. 4.—Relative rate and total percentage germination at 42° C. of *Amaranthus retroflexus* seeds collected at Pullman, Washington, in 1915: (*A*) untreated before being set to germinate; (*B*) treated with concentrated H_2SO_4; (*C*) ground with sand six weeks; (*D*) ground with sand nine weeks.

relation to temperature, and with those given by BALLS for rate of growth of sore-shin fungus in relation to temperature. Omissions in the tables of coefficients are due to lack of comparative data at certain temperatures.

Figs. 1, 2, and 3, included as representative graphs, show the difference in total percentage germination of seeds collected at the same time in one locality, and germinated at the same time at the same temperature but with seed coat intact in one lot, abraded by grinding with sand in another, and carbonized with H_2SO_4 in the third lot. This difference in total germination is greatest at low temperatures. Nevertheless, the coefficients for rate of germination computed from these curves in all lots run remarkably similar (tables I–III).

Fig. 4 shows the striking restricting effect of the coat at high temperatures, an effect paralleling the magnitude of the restricting effect of this coat at temperatures near the minimum temperature for germination. The removal of coat effects, either by grinding the seeds with sand or by treating with H_2SO_4, allows much more rapid germination at the same high temperature (42° C.) for the Washington seeds. Effects somewhat paralleling these were obtained for Indiana seeds at 46.1° C. In fig. 4 the effect of grinding the seeds with sand nine weeks is shown to be greater than the effect of grinding for six weeks. Concomitant with this slowness in germinating is the lack of anthocyanin in the seedlings developed at these high temperatures, and again apparently lessened amount in the seedlings developed at the lowest temperatures at which germination took place at all.

Conclusions

1. The coefficients relating rate of germination of seeds of *Amaranthus retroflexus* to temperature grade from high values as 10.01 (table II) at low temperatures, to low values as 0.001 (table II) at high temperatures, thus paralleling the coefficients relating rate of growth of seedlings and sore-shin fungus to temperature worked out by LEITCH, LEHENBAUER, and BALLS.

2. The general trend of these coefficients is the same for seeds only partially after-ripened, and for those with coat effects almost

completely removed by treatment with H_2SO_4 or abrasion with sand, as for instance 11.75 to 1.33 for Washington seeds untreated, and 5.77 to 1.37 for these seeds when treated with H_2SO_4 for three minutes.

3. In after-ripened seeds with coats untreated, the restricting effect of the coats shows particularly at low temperatures 8°–10° and 11.6°, and again at high temperatures, 42° for Washington seeds, and 46.1° for Indiana seeds. In both cases these effects can be lessened by treating the coats with H_2SO_4 or abrading them with sand.

Acknowledgment is due Dr. WILLIAM CROCKER for suggesting this problem, and guiding the work.

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NITROGEN FIXATION IN ERICACEAE

M. CHEVELEY RAYNER

(WITH FOUR FIGURES)

Introductory

Since the middle of the nineteenth century it has been known that plants belonging to the Ericaceae form mycorhiza of a characteristic kind. Further knowledge of the relations between plant and endophyte in this group has only recently been forthcoming.

In 1915 RAYNER[1] showed that the relationship in *Calluna vulgaris* is of a remarkable character, involving obligate symbiosis between the two organisms and a much more extensive distribution of the fungus throughout the green plant than had been suspected. As in Orchidaceae, root formation by seedlings is dependent upon early infection by the endophyte, failing which, development ceases and the plant perishes in the seedling stage. Unlike the condition in Orchidaceae, infection at the appropriate moment is provided for by the presence of mycelium on the seed coat, a condition ensured by the distribution of the endophyte throughout the vegetative tissues and eventually within the ovary chamber. These facts have been demonstrated with certainty in *Calluna*, and the evidence points to a similar condition throughout the family. Thus ovarial infection has been reported for many species in all the suborders of Ericaceae, and the inability of seedlings to complete their development without infection has already been proved for a number of these.

In such remarkable associations between flowering plants and fungi as are found in the orchids and in Ericaceae, it is of great interest to learn the exact nutritive relations between the symbionts. In orchids there is ocular evidence of digestion of mycelium by the cells of the root, and it is clear that by this means the plant can draw indirectly upon organic compounds of carbon and nitrogen in the soil. In the chlorophyllous orchids the endophyte can utilize

[1] RAYNER, M. C., Obligate symbiosis in *Calluna vulgaris*. Ann. Botany **29**:97–153. 1915.

the products of photosynthesis, but in the nonchlorophyllous forms, such as *Neottia* and *Corallorhiza*, this is not so, and, on the observed facts, the mutual relationship appears to be one of parasitism on the part of the green plant. Indeed this condition has fully been demonstrated for *Gastrodia elata*, a remarkable non-chlorophyllous species found in Japan. It is certain, therefore, that one at least of the so-called "saprophytic" orchids is parasitic upon a fungus, *Armillaria mellea*, and that the establishment of this relation has become obligate for the full development of the plant. This is the more interesting in that the fungus concerned is parasitic in habit and invades the tuber of the orchid in the first instance in exactly the same manner as it attacks the tubers of potato, upon which it is commonly found as a parasite in Japan.[2] In orchids the fungi endophytic in the roots do not spread into the chlorophyllous tissues, nor is there any evidence that they can use atmospheric nitrogen.

In *Calluna* the evidence as to exchange of food materials between the two partners may be summarized as follows. There is no indication of digestion of mycelium by the root, nor are there any obvious symptoms of attack or defense beyond the fact that hyphae effect an entry in the first instance and spread from cell to cell. That this vegetative activity depends upon a supply of food drawn from the plant cells rather than from organic compounds in the soil, is suggested by the normal behavior of the symbionts when grown in solutions of inorganic salts in pure culture. In the shoot, active mycelium is not readily demonstrated although widely distributed in a reduced condition; active hyphae occur in the extensive air spaces of the leaves, and grow into the air from the surface of the shoot. Moreover, there is evidence that mycelium undergoes digestion by the mesophyll cells of the leaf, and also that the fungus can hydrolyze arbutin outside the plant (RAYNER, *loc. cit.*). With respect to nitrogen assimilation, there is cumulative evidence that the endophyte of Ericaceae can utilize atmospheric nitrogen in greater or less degree, and it is the purpose of this paper to present this as briefly as possible.

[2] KUSANO, S., *Gastrodia elata* and its symbiotic association with *Armillaria mellea*. Jour. Coll. Agric. Tokyo 4:1–66. 1911.

The experimental evidence in question is derived from three sources. (1) The work of TERNETZ,[3] who showed that certain fungi isolated from the roots of ericaceous species could utilize atmospheric nitrogen. (2) The work of the writer (*loc. cit.*), which supplied the necessary link connecting these fungi directly with Ericaceae, and also provided additional evidence of the ability of certain ericaceous species to utilize atmospheric nitrogen. (3) The work of DUGGAR and DAVIS,[4] who undertook a critical experimental review of the difficult problem of nitrogen fixation by fungi. The evidence provided by these workers will now be considered in historical sequence.

(1) The researches of TERNETZ were undertaken in connection with an attempt to isolate the root endophytes of Ericaceae, concerning which no information was at that time available. As a result of prolonged experiments, eight pycnidia-forming fungi were isolated, five of which were investigated for evidence of fixation of gaseous nitrogen. All forms isolated were referred by LINDAU and HEMMINGS to *Phoma*, and differed in the small size of the pycnidiospores (4–5 μ in length) from the species previously found associated with Ericaceae. The five forms experimented with were isolated from the roots of *Oxycoccus palustris*, *Andromeda polifolia*, *Vaccinium Vitis-Idaea*, *Erica Tetralix*, and *E. carnea*, and were named *Phoma radiciis Oxycocci*, *P. radiciis Andromedae*, *P. radiciis Vaccini*, *P. radiciis Tetralicis*, and *P. radiciis Ericae*, respectively. TERNETZ has put on record the interesting observation that these fungi, although isolated from plant species growing in close proximity, are specific strains, distinguishable by definite morphological and physiological characters.

The isolation of fungal species endophytic in the roots of plants is a matter of notorious difficulty, and their identity can only be proved by formation of mycorhiza typical for the species following upon inoculation from pure culture into the roots of seedlings free from fungal infection. Those isolated by TERNETZ were

[3] TERNETZ, C., Über die Assimilation des atmosphärischen Stickstoffes durch Pilze. Jahrb. Wiss. Bot. **44**:353–408. 1907.

[4] DUGGAR, B. M., and DAVIS, A. R., Studies in the physiology of the fungi. I. Nitrogen fixation. Ann. Mo. Bot. Gard. **3**:413–437. 1916.

believed to be the endophytes associated with the different species, but the necessary proof was lacking, inasmuch as seedlings of the latter were never obtained free from mycorhiza. All attempts to sterilize seeds failed, since sooner or later the roots of seedlings raised from such seeds showed the mycorhizal condition typical of the species in nature. The five strains of *Phoma*, as well as *Aspergillus niger* and *Penicillium glaucum*, were subsequently cultivated on media free from combined nitrogen. The cultures were carried on over a period of several years, were frequently repeated, and due precautions were observed with regard to purity of materials, adequacy of controls, and methods of estimation. It is recorded that none of the fungi investigated required a supply of combined nitrogen for healthy development or growth. They all fixed atmospheric nitrogen, but in very different degrees. The values

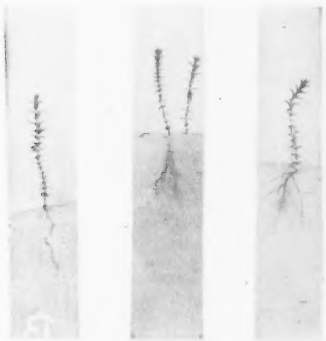

FIG. 1

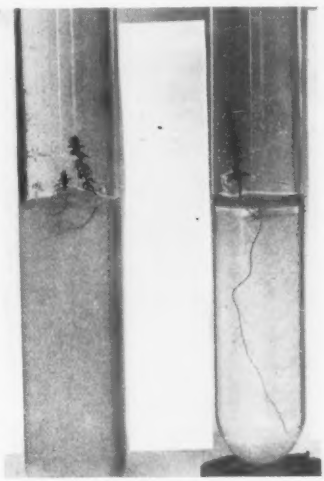

FIG. 2

FIG. 1, 2.—Fig. 1, *Calluna vulgaris:* representative seedlings (four months old) from large number grown in agar nutrient lacking nitrates; growth continued until root system occupied whole of rooting medium; shoots averaged 3.5–5 cm. length; fig 2, *Calluna vulgaris:* representative seedlings (three months old) from silica jelly cultures lacking combined nitrogen; silica nutrient in right-hand tube chanced to be more liquid in consistency than other tube, or than tubes shown in fig. 3, hence more vigorous root development; finer roots in upper part of root system not visible in photograph.

obtained for *Aspergillus* and *Penicillium* are too small to have any critical value, and agree in this respect with those of previous investigators. The highest capacity for nitrogen fixation was found in the strains of *Phoma* isolated from *Oxycoccus*, *Vaccinium*, and *Andromeda*, in which the values cited appear to be well outside the range of any possible experimental error. Table I, reproduced from the original paper by TERNETZ, shows the values obtained for *Phoma radiciis* as compared with those on record for the nitrogen-fixing bacteria.

TABLE I

RECORDS OF NITROGEN FIXATION BY FUNGI EXTRACTED FROM FOUR ERICACEOUS SPECIES, AS COMPARED WITH THAT EXHIBITED BY *Clostridium*, *Azotobacter* *Aspergillus*, AND *Penicillum*, FROM TERNETZ (*loc. cit.*).

ORGANISM	DAYS	DEXTROSE SUPPLIED		DEXTROSE USED (GM.)	NITROGEN FIXATION		INVESTIGATOR
		gm.	Per-centage		mg.	per gm. dextrose (mg.)	
Clostridium Pasteurianum..	20	40	4	40	53.6	1.34	Winogradski
Clostridium Pasteurianum..	20	20	2	20	24.4	1.22	Winogradski
Clostridium americanum...	30	1.25	0.25	1.25	4.6	3.7	Pringsheim
Clostridium americanum...	30	5	1	3.01	8.2	3.01	Gerlach and Vogel
Azotobacter chroococcus...	35	5	0.5	5	42.7	8.56	Gerlach and Vogel
Azotobacter chroococcus...	35	12	1.2	12	127.9	10.66	Ternetz
Aspergillus niger.....	28	7	7	1.1	1.9	1.71	Ternetz
Penicillium glaucum.....	28	7	7	0.7	2.8	3.8	Ternetz
Phoma radiciis Oxycocci...	28	7	7	0.85	15.3	18.08	Ternetz
Phoma radiciis Andromedae	28	7	7	0.67	7.3	10.92	Ternetz
Phoma radiciis Vaccinii....	28	7	7	0.71	15.7	22.14	Ternetz
Phoma radiciis Tetralicis...	28	7	7	1	4	3.99	Ternetz
Phoma radiciis Ericae.....	28	7	7	1.1	2.3	2.17	Ternetz

The three fungal strains concerned work much less energetically but more economically than *Clostridium* or *Azotobacter*. For example, for each gram of dextrose used, 22 mg., 18 mg., and 11 mg. of nitrogen were combined, as compared with values ranging from 1.2 mg. to 10.6 mg. of nitrogen per gram of dextrose for the nitrogen-fixing bacteria. These are the highest relative figures on record for nitrogen-fixing organisms.

(2) The evidence contributed by the writer was obtained in the course of an intensive experimental study of *Calluna vulgaris*, and supplied the link needed to connect the fungi isolated by TERNETZ

with their ericaceous host plants. In ignorance of the work of TERNETZ, the conclusion was reached independently that seedling roots become infected from the testa subsequent to germination. This view proved to be correct, and a pycnidia-bearing fungus was eventually isolated with comparative ease from unopened fruits. Proof of the identity of this fungus was then provided by reinoculation into seedlings grown in pure culture and raised from sterilized seeds. A remarkable condition of obligate symbiosis was thus put on record for *Calluna*, and the observations made by TERNETZ as to the specificity of the fungal strains in the different ericaceous species were subsequently confirmed. The characters of the endophyte isolated from *Calluna* agree with those described by TERNETZ, and the necessary proof is thus provided that the forms experimented with by this worker were actually those associated with the five plant species concerned. In view of this fact, the suggestion previously put forward as to nomenclature (*loc. cit.*, p. 125) should be withdrawn and the name *Phoma radiciis Callunae* accepted.

In the paper recording these facts, attention was drawn to observations bearing on the possibility of nitrogen fixation by the endophyte. Of these may be mentioned: (1) the vigor and longevity of seedlings germinated on filter paper moistened with distilled water (RAYNER[5]); (2) the wide distribution of the endophyte throughout the plant tissues, its development in the intercellular spaces of the leaves and emergence to the air from the surface of the shoot; (3) the evidence of digestion of mycelium by mesophyll cells (see footnote 1). The association of *Calluna* and other ericaceous species with soils deficient in nitrates in itself provides *raison d'être* for the remarkable biological relations between plant and fungus, assuming fixation of atmospheric nitrogen on the part of the latter.

Experimental observations

CALLUNA SEEDLINGS IN MEDIA LACKING COMBINED NITROGEN.—In experimental cultures, seedlings of *Calluna* grow readily under aseptic conditions in a dilute normal solution made with 1.2 per cent agar-agar. In order to test the possibility of cultivation in a

[5] RAYNER, M. C., The ecology of *Calluna vulgaris*. New Phytol. 12:59–77. 1913.

substrate free from combined nitrogen, pure culture seedlings were planted in a similar medium lacking nitrates, both sets of seedlings being infected from a pure culture of the endophyte at planting. These cultures were first grown in 1915, and no special precautions were observed beyond the use of pure chemicals and freshly distilled water. The seedlings not supplied with nitrate grew surprisingly well. They

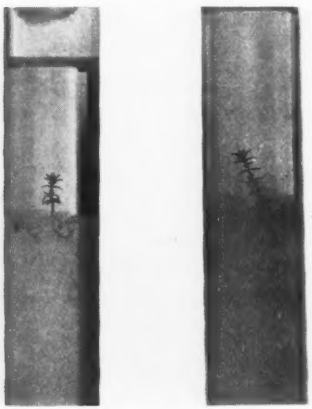

FIG. 3

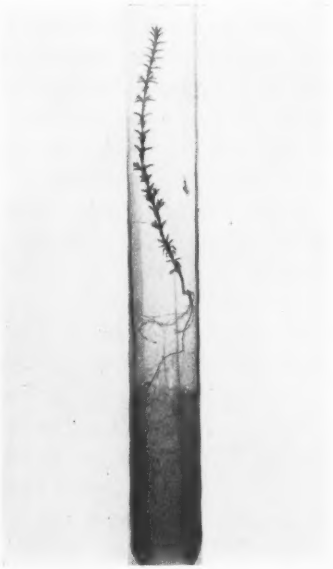

FIG. 4

FIGS. 3, 4.—FIG. 3, control seedlings planted simultaneously with those in fig. 2 in silica jelly nutrient containing combined nitrogen in form of KNO_3; fig. 4, same seedling as shown in right-hand tube of fig. 2, five and a half months old; shoot reached height of over 6 cm.

were, on the average, healthier than the controls, a brighter green, and of quite as vigorous growth. The controls were supplied with potassium nitrate in the proportion of 0.5 gm. per liter. They showed no differentiating features and unfortunately were not photographed. It should perhaps be mentioned that the cultures were grown in a small cold greenhouse away from the laboratory. Kjeldahl estimations of samples of the agar medium subsequently made yielded negative results.

It had been observed previously that *Calluna* seedlings thrive only in solutions of extremely low total concentrations of salts (0.05 per cent). The experiments just described show further that in cultures of "synthetic" seedlings, the use of a culture fluid of 0.05 per cent total concentration affords optimum conditions for growth.

The experiments have since been repeated, using every possible precaution to avoid contamination by traces of combined nitrogen. A similar solution of inorganic salts was made up in silica jelly prepared from specially purified materials and ammonia-free water. The cultures were planted in the autumn under unfavorable weather conditions, and seedlings did not root freely in the silica jelly, which seemed to offer mechanical difficulties. Otherwise, the results confirmed those already described. The seedlings deprived of combined nitrogen were green and healthy and grew at the same rate as the controls.

It may be objected that seedlings of *Calluna* could grow for several months on the seed reserves, and that this accounts for the vigor and longevity shown by seedlings supplied with distilled water only. Against this interpretation is the fact that seedlings germinated on moist filter paper from sterilized seeds not only form no roots, but make practically no shoot growth and quickly show symptoms of starvation such as yellowing and discoloration of the leaves. These symptoms are relieved by inoculation from a pure culture of the endophyte. Finally, there can be no doubt that the optimum conditions in artificial cultures for the establishment and maintenance of a properly balanced relation between plant and endophyte are supplied by a rooting medium of extremely low concentration of salts (for example, 0.05 per cent) lacking combined nitrogen. A fresh line of research is hereby suggested in order to ascertain whether the unfavorable symptoms shown by seedlings planted in culture solutions of higher concentration of salts can be specially correlated with the supply of nitrates. It is certain that a very small alteration in the character of the nutrient supplied to "synthetic" seedlings overthrows the normal balance and induces parasitism in the endophyte.

(3) Additional evidence on nitrogen fixation in Phoma.—An indirect contribution to the subject has recently been made by

DUGGAR (*loc. cit.*). In the course of an experimental review of previous work on nitrogen fixation by fungi, DUGGAR has repeated and extended the observations of earlier workers on this subject, taking extraordinary precautions to avoid experimental methods open to criticism on the score of inaccuracy. Among the species thus investigated are *Penicillium* spp., *Aspergillus niger*, *Macrosporium commune*, *Glomerella Gossypii*, and *Phoma Betae*, as well as three forms of *Azotobacter* isolated from different soils. With regard to the four first named genera, DUGGAR'S work confirms that of previous observers, namely, that these fungi can utilize atmospheric nitrogen to a very slight extent. The amounts recorded are very small, and in DUGGAR'S opinion cannot be accepted as conclusive evidence of ability to fix atmospheric nitrogen. On the other hand, the values obtained by DUGGAR for *Phoma Betae* range from 3.022 mg. to 7.752 mg. per 50 cc. of culture fluid, a known amount of combined nitrogen being supplied. These values are of special interest for comparison with those recorded by TERNETZ for the forms of *Phoma radiciis* extracted from the roots of ericaceous species. Indeed, the evidence appears to be conclusive that ability to continue to fix atmospheric nitrogen exists in varying degree in *Phoma*.

The experimental results obtained by the writer indirectly support this view, and provide a basis for an intelligible explanation of one physiological aspect of the relation between green plant and fungal symbiont in Ericaceae; incidentally, they throw light on the proved ability of species such as *Calluna* and *Vaccinium* to thrive in soils deficient in nitrates. The degree of nitrogen fixation by the endophyte doubtless varies with the species concerned, and may operate as an important survival factor for the plant growing under competitive conditions.

Summary

1. In 1907 TERNETZ provided evidence that certain strains of *Phoma*, isolated from the roots of ericaceous plants, could utilize atmospheric nitrogen.

2. In 1915 the necessary proof that the fungi extracted by TERNETZ were actually the endophytes was provided by the

writer, who showed also that seedlings of *Calluna vulgaris* in pure culture thrive in a rooting medium lacking combined nitrogen.

3. In 1916 DUGGAR offered additional evidence for fixation of nitrogen by members of *Phoma*.

The experimental work of which this paper gives an account has been in part carried out at the Pilcher Research Laboratory, Bedford College, University of London, with the aid of a grant from the Dixon Fund of the University of London.

BEDFORD COLLEGE FOR WOMEN
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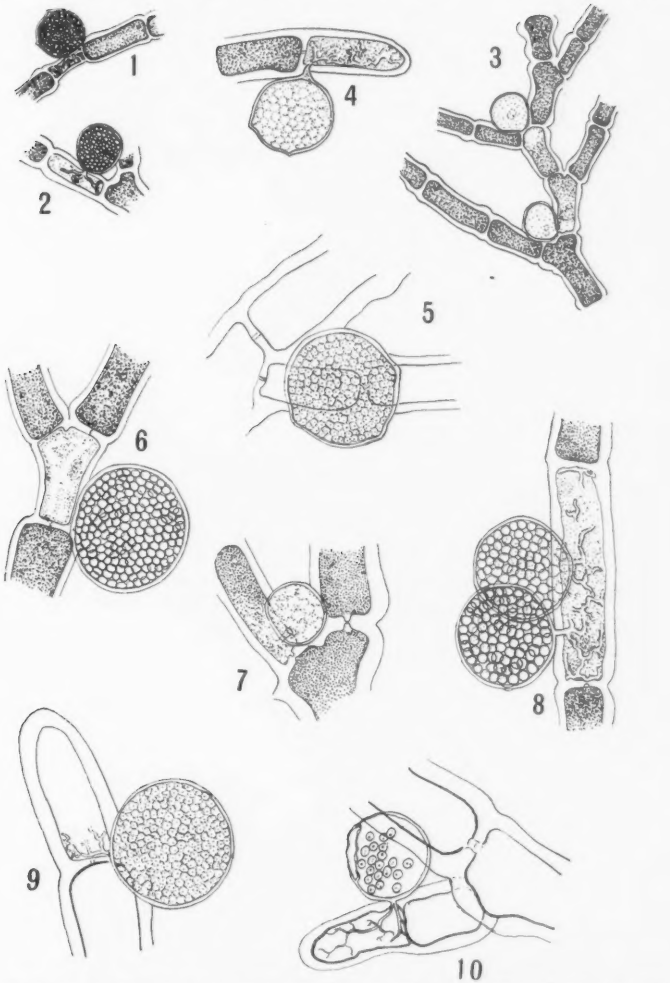
RHIZOPHIDIUM POLYSIPHONIAE IN THE UNITED STATES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 292

GEORGE W. MARTIN

(WITH TEN FIGURES)

The morphology and distribution of the fungi parasitizing marine plants are still so imperfectly known that additional facts concerning any of them seem worth placing on record. Among some algal material collected by Dr. T. C. NELSON in Barnegat Bay, New Jersey, and sent by him to the writer preserved in formalin, occurred a small sterile plant of *Callithamnion* which was observed to bear numerous sporangia of a chytridiaceous fungus. The mature sporangia were globose or nearly so, from 22 to 39 μ in diameter, averaging 33 μ, and closely appressed to the attacked host cell, which could be sharply distinguished from the neighboring unattacked cells by the partial or nearly complete exhaustion and decoloration of its contents. In only one instance was more than one sporangium attached to a single host cell (fig. 8). The cell contents are destroyed first at the end at which the fungus is attached (fig. 3), and by the time the sporangium becomes mature the contents of the parasitized cell are, as a rule, almost exhausted (figs. 1, 2, 6, 8). In some cases a branched, rootlike mycelium, rather coarse for this genus, could be seen within the host cell and traced to the base of the sporangium (figs. 1, 2, 8, 9, 10). More frequently the mycelium could not be distinguished. The zoospores are from 2 to 3 μ in diameter, globose or somewhat irregular in shape, and are evidently liberated through an opening developed from a papilla, of which each sporangium bears from one to several (figs. 1, 4, 5). Each zoospore contains a nucleus or oil globule, and in addition a much smaller body, observed only in the spores remaining in a nearly



FIGS. 1–10.—Figs. 1, 2, mature sporangia, one with two papillae, showing mycelium in host cell; fig. 3, two immature sporangia showing exhaustion of host cells at end of which sporangia are attached; fig. 4, immature sporangium with two papillae; fig. 5, nearly mature sporangium from above, with three papillae (sporangium attached to basal cell of smaller branch); figs. 6, 9, mature sporangia, latter showing mycelium; fig. 7, immature sporangium, showing rootlike base; fig. 8, two mature sporangia on same host cell; fig. 10, nearly empty sporangium; spores with nuclei and blepharoplasts. Figs. 1–3, ×480; figs. 4–10, ×1000; all reduced to one-half in reproduction.

empty sporangium (fig. 10), presumably a blepharoplast. The wall of the sporangium is smooth and firm and about 1 μ in thickness.

According to the classification of SCHRÖTER[1] this fungus belongs to the genus *Rhizophidium*, of which several species have been reported as attacking hosts which occur in salt or brackish water. Of these, only *R. polysiphoniae* (Cohn) Petersen resembles the species under consideration. This fungus was originally described from Helgoland by COHN[2] under the name *Chytridium polysiphoniae*, as lacking a mycelium and with a dark colored membrane and a definite operculum, although the latter is not shown in COHN's later figure.[3] PETERSEN[4] redescribed the species as a *Rhizophidium*, stating that it possessed a definite mycelium, and, except in old specimens, a hyaline membrane, and reports it as occurring in several localities in Denmark on *Polysiphonia*, *Ceramium*, *Delessaria*, and *Callithamnion*. The correspondence between this species and the form from New Jersey is not complete, since in the latter no suggestion of a definite operculum could be seen, and some of the sporangia had at least three, possibly more, papillae. Nevertheless, the resemblance between the New Jersey species and the descriptions and figures of COHN and PETERSEN is so close that it seems inadvisable, without studying living material, to regard them as distinct.

UNIVERSITY OF CHICAGO

[1] SCHRÖTER, J., Chytridineae. In ENGLER and PRANTL, Die Natürlichen Pflanzenfamilien 1:[1] 1892.

[2] COHN, FERDINAND, Chytridii species novae marinae. Hedwigia 4:169–170. 1865.

[3] ———, Beiträge zur Physiologie der Phycochromaceen und Florideen. Archiv Mikr. Anatomie 3:1–60. 1867.

[4] PETERSEN, H. E., Contributions a la connaissance des Phycomycetes marins (Chytridinae Fischer). Overs. Danske Videnskabernes Selskabs. Forh. 1905:439–488.

CURRENT LITERATURE

BOOK REVIEWS

Transpiration of plants

BURGERSTEIN'S[1] second volume on the transpiration of plants constitutes a supplement to his well known work published in 1904. It presents a critical summary of the literature on transpiration down to and including the year 1920. The first volume was based on 394 publications, while in the second volume 505 have been added, many of which were published in English, and an unusually large number by women. The first volume contained 30 chapters, the second 32. They parallel each other very closely, but a comparison of the two brings out rather clearly the more recent trend of this line of investigation. Many of the subjects mentioned in the text in the first volume are here given separate chapter treatment. Thus the principal advances made in the study of the transpiration of plants during the last sixteen years are clearly indicated. A number of new terms and phrases appear in the second volume which were not used in the first, and most of which are concerned with an attempt on the part of the investigator to obtain a more satisfactory basis for the comparison of the amount of transpiration of different plants at different times and under different conditions.

Relative transpiration is the ratio of transpiration of any plant at any time to the water loss from a standardized water surface, or from any other water-evaporating surface exposed under the same conditions as is the plant, and for the same length of time. In most of the papers in which this term is used the assumption has been made that evaporation is a correct measure of the environmental conditions affecting transpiration, and that therefore any variation which occurs in the plotted graph showing ratio of transpiration to water loss is due to some adjustment on the part of the plant. This assumption is made without sufficient reason and is not well supported by experimental data.

Index of transpiring power differs from relative transpiration only in that a cobalt paper is introduced as an indicator of relative water loss; consequently any errors inherent to the relative transpiration method are not eliminated by this method, and there is also introduced the uncertainty connected with the use of the cobalt paper. Both of these methods have been used extensively and have stimulated an unusually large amount of investigation. While the accuracy of the results must be questioned, it is undoubtedly true that our knowledge of transpiration has been greatly advanced by their application.

[1] BERGERSTEIN, ALFRED, Die Transpiration der Pflanzen. Zweiter Teil (Ergänzungsband). Jena. 1920.

Specific transpiration is really an expression of the rate of drying of plants. It is the percentage of the total water content of the plant lost during a definite time. The loss would naturally be very high in plants with low water content and very low in plants such as succulents, which have very high water content.

Correlative transpiration has been used in the sense of relative transpiration, and also to express the inter-relationship between transpiration of leaves in the shade and in the sun, bringing out the fact that leaves in the sun will often withdraw the water from the leaves in the shade, and although their transpiration is much more rapid than leaves exposed in the shade, will continue fresh while those in the shade wilt.

Water requirement is used in two different ways. In the broadest application of this word it is synonymous with the expression "water relation of plants," but by many it has been used in a narrow sense to signify the ratio of water consumption to dry matter produced during the growth of a plant.

Many new methods have been employed, both for determining the stomatal openings and measuring the water loss. The epidermis has been fixed either in alcohol or picric acid and the openings measured by microscopic examination. The rate of the flow of air through the leaf, or the porometer method, which has several modifications, has enabled the experimenter to estimate the relative difference in the openings of the stomata. To this method has also been applied automatic recording devices.

One of the simplest and most useful methods for determining whether or not the stomata are open is the infiltration method. Absolute alcohol, petroleum ether, or other fluids, when dropped on a leaf with open stomates penetrate into the mesophyll. This penetration is easily observed, and the technique is so simple that observations can be made rapidly.

Several modifications of the gas diffusion or gas infiltration method have been employed by different workers. A large variety of potometers and atmometers have been devised. Nothing especially new has appeared among the porometers, but a number of new types of atmometers have been employed. These consist chiefly of porcelain filters or of filter paper saturated with water which is allowed to evaporate and the loss determined by weighing.

In the measuring of transpiration loss no entirely new methods have been devised, although great improvements have been made on the older methods. Automatic weighing devices have been greatly improved, and there has been considerable improvement also in the methods of measuring transpiration by collecting the transpired water. There are now several types of automatic instruments, chiefly of the step-by-step type, which give satisfactory records of transpiration loss.

Efforts to find algebraic equations or formulae by which transpiration can be estimated from the observed environmental conditions, has resulted in a clearer understanding of the factors affecting transpiration, but no entirely satisfactory equations have been deduced. In fact, experimental data are hardly sufficient at the present time to enable one to evaluate properly such factors as wind, light, etc., and this, combined with the uncertainty of the

effect of stomatal movement, makes the problem an especially difficult one. It is true, however, that the effect of external conditions on transpiration, determined usually by comparing transpiration at different places at different portions of the day or year, is now much better understood than at the time of publication of the first volume. These results have been obtained usually with the help of either painstaking direct weighings, or by the use of automatic transpiration records, the evaporation, temperature, sunlight, wind, and wet bulb depression having been simultaneously determined.

Water requirement of plants used in its narrow sense is the amount of water consumed by a plant during its period of growth in the production of a unit weight of dry matter. It is evident, therefore, that any factor which affects transpiration and any factor which affects the growth of plants will modify this ratio. Where conditions are most favorable for growth the water requirement is likely to give the lowest value, while if conditions are not favorable for growth, even though the transpiration rate be relatively low, the water requirement will still be high. The practical value of this measurement in connection with the production of cultivated crops has led to a large number of determinations covering many of the more important crop plants. The relative consumption of water during growth and the effect of environmental conditions on the water requirement are less easily determined than the effect of environmental conditions on transpiration, since not only do the conditions control the rate of transpiration, but also affect the relative rate of growth for production of dry matter.

Although very little work has been done on the effect of insufficient soil moisture on the transpiration of plants, a great mass of data has been accumulated on the amount of moisture in the soil at the time plants wilt. Although results are somewhat conflicting, it has been found that there is a relatively definite percentage of soil moisture content, beyond which the movement of moisture in the soil is so slow as to make it practically impossible for a plant to supply its transpiration demand from the mass of soil through which its roots ordinarily extend. This moisture content has been referred to as the wilting coefficient.

Many papers have dealt with structure and morphological investigations for lessening transpiration. Although the value of transpiration in reducing the temperature of leaves has been brought out by a number of investigators, its value in relation to the nutrition of plants has not fully been admitted.

The difficulty of getting together so large a volume of English literature, especially at a period when war made access to literature from English speaking countries difficult or impossible, must have been very great. This work is a valuable summary, and no investigation of transpiration is feasible without first consulting it. It is impossible to bring into the work all of the material contained in the original papers, and these should always be consulted. A careful perusal of this work shows clearly that there is not a single line of investigation at the present time which does not afford a good starting point for further research.—H. L. SHANTZ.

Lake Maxinkuckee

The Department of Conservation of the state of Indiana has recently published a remarkable monograph on Lake Maxinkuckee, a physical and geological survey by EVERMANN and CLARK.[2] Lake Maxinkuckee is a small glacial lake similar to thousands of other lakes in the northern Mississippi Valley states, and because of this similarity any study of Lake Maxinkuckee or conclusions drawn therefrom would be typical of other lakes in this region. The work was begun and chiefly financed by a bureau of the United States government, now known as the Bureau of Fisheries. There is a general feeling of surprise and disappointment that the results were not published by the United States government. The Department of Conservation of Indiana, however, is to be congratulated, not only for the excellent manner in which the monograph is published, but also for having saved it from the shelves of discarded manuscripts. The monograph is more than a simple study of the lake, for it treats of the animal and plant life of the lake, as well as the physical surroundings and the life around it and in the air above it. It gives a vivid presentation of the physical, hydrographic, and meteorological features which belong to the lake, and a record of the animal and vegetable life in and about it.

The physical conditions discussed relate to the location, altitude, size, and form of the lake, and the character of the surrounding country. A list of the streams which feed the lake is given, as well as a most interesting account of the ice beach. Under hydrography is discussed the depth of the lake, the topography and character of its bottom, with special discussion of certain deep places, its level, the stage of water, and the volume of outflow. Under meteorology is discussed the sky, the air, the pressure and temperature, the winds, rain, frost, and even the snow, fog, and dew. Then follow exhaustive tables relating to the water temperature and the condition of the water and the formation of the ice. The most interesting paragraph in this chapter relates to the turning over of the lake each fall. Although this phenomenon was not actually observed, it was shown by an elaborate series of soundings just when the change must have taken place. Emphasis, however, is given to the biological features, to which two-thirds of the first volume and all of the second are devoted. The largest part of the biology of course is given to the fishes, since this was the primary object of the investigation. It is stated that 64 species were found in Lake Maxinkuckee. These are all described fully, with many interesting notes on their habits and food value, while there are many suggestions about angling. Many of the fishes are illustrated in color. Thirty species of mammals are recorded, and there are interesting notes about all of them, especially the two species of wolves which once inhabited this part of Indiana, and the disappearance of the beaver and porcupine is noted. Much attention is given to the birds and especially

[2] EVERMANN, B. W., and CLARK, H. W., Lake Maxinkuckee, Vol. 1. pp. 660. *pls.* 36. *fig.* 23. Vol. 2. pp. 512. 1920.

to the water fowl, and 175 species are recorded. Considerable space is given to insects and the lower forms of animal life, and especially to those which are of value to the fish fauna of the lake.

The larger part of the second volume is devoted to the flora of Lake Maxinkuckee and its vicinity. This includes a special chapter on the aquatic flora and its uses, and a chapter on the algae, of which 76 species are mentioned. The volume closes with an annotated list of the ferns, fern allies, and seed-bearing plants found in the lake and the surrounding basins, of which 838 species are listed. The arrangement and nomenclature is that of the second edition of BRITTON and BROWN'S *Illustrated Flora*. The remarks about the various plants are very readable, while some of the observations are quite unique and have heretofore been unrecorded.

Besides the scientific value of the monograph, its important educational value should not be overlooked. Dr. EVERMANN, the senior author, although now a well known scientist, was originally a teacher in our elementary schools, and the educational importance of scientific research has always been emphasized by him. This book should be made a most helpful guide to the science teachers in our high schools and colleges who wish to do field work. It forms a model for the study of the lake or river valley or even the pond or creek in one's own locality. If the science teachers in the ninety-two counties of Indiana alone should use it with their classes in the study of local problems, a mass of information about the state would be accumulated, and a wonderful interest in nature study would be developed.—J. N. ROSE.

NOTES FOR STUDENTS

Taxonomic notes.—The vascular plants collected by the Canadian Arctic Expedition of 1913–18 on the Arctic coast west of the 100th meridian have been published by MACOUN and HOLM,[3] the latter completing the determinations after the death of MACOUN. There have also been included three other collections from the same region. The enumeration includes 230 species, Compositae including 23, Gramineae 22, Ranunculaceae 19, Cruciferae and Saxifragaceae each 18, etc. The largest genus represented is *Saxifraga* with 15 species, followed by *Carex*, *Salix*, and *Ranunculus* each with 12 species. Some interesting comparisons are made with the flora of Greenland and of the west coast of Alaska.

EVANS[4] has published a detailed study of the liverwort genus *Riccardia*, "often known as *Aneura*," as it is represented in Chile. He recognizes 25 species, of which 3 are new and 17 new combinations. The descriptions are very full, so that the presentation is morphological as well as taxonomic.

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[3] MACOUN, JAMES M., and HOLM, THEO., Report of the Canadian Arctic Expedition 1913–18. **5**: Part A. 1–51. *pls.* 13. 1921.

[4] EVANS, A. W., The genus *Riccardia* in Chile. Trans. Conn. Acad. Sci. **25**: 93–209. *figs.* 13. 1921.

SCHLECHTER,[5] in monographing the tribe Thismieae of Burmanniaceae, recognizes ten genera, the following two being new: *Scaphiophora* and *Triurocodon*.

PERKINS,[6] in monographing the African species of *Pycnostachys* (Labiatae), recognizes 33 species, 8 of which are new. The same author has also monographed the African species of *Achyrospermum* (Labiatae), recognizing 12 species, 3 of which are new.

BROWN,[7] in naming a collection of plants from southeastern Congo, Rhodesia, and South Africa, has described 30 new species, and also a new genus (*Alistilus*) of Leguminosae.

DIELS,[8] in continuation of his investigation of the flora of Micronesia, has published the following families: Myrtaceae, Myrsinaceae, Elaeocarpaceae, Asclepiadaceae, Scrophulariaceae, and Gesneraceae.—J. M. C.

Citrus diseases in the Orient.—The study of citrus diseases in the Orient is of particular interest and importance since most of our cultivated citrus fruits undoubtedly had their origin in this region. REINKING'S[9] recent paper therefore, is timely and interesting. A description of the diseases, a discussion of the causal organism, and suggestions regarding the control measures proper for each is given. A summary showing the citrus varieties found in each country, with the diseases to which they are subject, is given, also a list of scale insects and fungi parasitic on scales. Fourteen good plates, devoted chiefly to illustrating citrus canker (*Pseudomonas citri*), bark rot (*Diplodia*), and pink disease (*Corticium salmonicolor*) complete the article. The two latter diseases, occurring in the Philippines and unknown in the United States, are apparently of major importance, warranting every precaution against their spread or introduction into new territory. A "black spot" disease occurring in South China, of unknown cause, is also regarded as serious.

Particular attention is given to the degree of susceptibility to citrus canker shown by different species, hybrids, and relatives of citrus planted out at Los Baños, Philippine Islands. Observations of this character have an important bearing on the selection of material for culture in regions exposed to canker

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[5] SCHLECTER, R., Die Thismieae. Notizblatt Bot. Gart. u. Mus. Berlin-Dahlem. **8**:no. 71. 31–45. 1921.

[6] PERKINS, JANET, Die afrikanischen *Pycnostachys*-Arten. Notizblatt Bot. Gart. u. Mus. Berlin-Dahlem. **8**:no. 71. 63–77. 1921; Die afrikanischen *Achyrospermum*-Arten. *Ibid.* 78–82.

[7] BROWN, N. E., New plants from tropical and South Africa collected by Archdeacon F. A. ROGERS. Kew Bull. 1921:no. 8. 289–301.

[8] DIELS, L., Beiträge zur Flora von Mikronesien und Polynesien. II. Engler's Bot. Jahrb. **56**:529–577. 1921.

[9] REINKING, OTTO A., Citrus diseases of the Philippines, South China, Indo-China, and Siam. Philippine Agriculturist **9**:121–179. 1921.

infection. Hybrids having one or more resistant parents show in many instances promising resistance. The discussion, representing as it does some forty diseases and pests in the Philippines and nearly an equal number in the Asiatic countries visited, emphasizes the need for intensive studies of plant diseases in the regions where they have been long established.—W. T. SWINGLE.

Stelar morphology.—In his presidential address to the Royal Society of Edinburgh, BOWER[10] emphasizes the importance of the principle of similitude (GALILEO) in the investigation of the stelar morphology of the higher plants. He argues that, inasmuch as the surface of an organ or tissue varies only as the square of its linear dimensions, but the bulk as the cube, the larger a plant is the more dependent it will be upon its form and detailed structure, not only for its stability, but also for the performance of its functions of absorption and transit of liquids and gases. This will apply not only to the external surface, but also to those internal surfaces which limit one tissue tract from another. Upon the basis of this premise, he concludes that in the ontogeny and phylogeny of ferns the form of the vascular tissues is largely dependent upon the size of the plant and of its various organs. Thus, as the fern plant and its foliar appendages become larger, the simple and presumably primitive protostele tends to become involuted, medullated (solenostely), or dissected into separate strands (polycycly, perforation, dictyostely).

BOWER'S correlations between size, form, and function are very suggestive, and deserve careful consideration, particularly by students of the phylogeny of the vascular cryptogams. It must be admitted, however, that there is a considerable element of uncertainty in interpreting such correlations. The fact that complex structures tend to occur in large plants does not prove necessarily that size is the primary factor in their evolution, although such a conclusion appears to be extremely plausible.—I. W. BAILEY.

Deccan vegetation.—The ecological problems of many portions of India are complicated by the density of the population and the intensity of the grazing. The rainfall of 27 inches in the Deccan coming during the months from June to October, preceded by a very hot and dry period, causes the erosion of fields denuded of vegetation by drought and grazing. In such a region the study of natural vegetation in areas protected from cattle has been begun by BURNS and CHAKRADEV[11] as a preliminary to work on the improvement of grazing lands. Permanent quadrats were established within barbed wire inclosures. Native grasses such as *Andropogon monticola* and *Iseilema laxum* appear to be able to establish themselves completely, and it seems

[10] BOWER, F. O., Size, a neglected factor in stelar morphology. Proc. Roy. Soc. Edinburgh **41**:1–25. 1921.

[11] BURNS, W., and CHAKRADEV, G. M., An ecological study of Deccan grassland. Jour. Indian Bot. **2**:84–91. 1921.

possible that a more mesophytic grassland may be the climax, with the formation of a turf resisting erosion.

Investigations by BHIDE[12] during one of the worst droughts on record, in 1918–19, have taken into account some of the plants showing the most successful resistance to such arid conditions. Such data not only add to our knowledge of the existing vegetation, but furnish material for improving existing economic conditions in a region where grazing is of first importance.

The anatomy of many plants of the arid region is also being investigated by SABNIS.[13] The results of such efforts are certain to be valuable for India and interesting to botanists elsewhere.—G. D. FULLER.

Tension zone between forest and prairie.—Following an earlier study by WEAVER and THIEL, an interesting tension zone investigation has been carried on by POOL, WEAVER, and JEAN[14] in eastern Nebraska. Stations were selected at Peru, near the Missouri River, and at Lincoln, sixty miles west of Peru. By means of quantitative experimental study, striking contrasts between these two stations, due to both climatic and edaphic factors, were brought to light. The prairies and woodlands near Lincoln are much more xerophytic than those near Peru, in spite of the short distances involved between the two places. Available soil moisture during the summer of 1917 was exhausted on eighteen different days on a Lincoln prairie and on only four different days on a comparable Peru prairie. Many mesophytic woodland species pass out in traversing the area between these two places. The high saturation deficit and the low soil moisture content of the prairie sites in eastern Nebraska constitute barriers over which forest trees can scarcely pass. The authors feel that herein is the most ready explanation for the confinement of Nebraska woodlands to the moist slopes of narrow valleys and for the general treelessness of prairies. In the order of increasing mesophytism, the forests about Peru are as follows: bur oak-yellow oak, black oak-hickory, red oak, linden-ironwood, while the common forest type about Lincoln is that of the bur oak-hickory.—H. C. COWLES.

Composition of plants as affected by nutritive elements.—Growing the oat plant in analyzed quartz sand, DICKSON[15] has made a study of the effects of a deficiency of certain nutrient elements on the calcium and phosphorus

[12] BHIDE, R. K., Drought resisting plants in the Deccan. Jour. Indian Bot. **2**: 27–43. 1921.

[13] SABNIS, T. S., The physiological anatomy of the plants of the Indian desert. Jour. Indian Bot. **2**:1–19, 61–79, 93–115. 1921.

[14] POOL, R. J., WEAVER, J. E., and JEAN, F. C., Further studies in the ecotone between prairie and woodland. Univ. Nebraska Studies **18**:1–47. *figs. 17*. 1918.

[15] DICKSON, J. G., The relation of certain nutritive elements to the composition of the oat plant. Amer. Jour. Bot. **8**:256–274. *figs. 2*. 1921.

content of the plant. The elements, the amount of which present in the nutrient solutions was varied, were calcium, magnesium, potassium, phosphorus, and nitrogen. The plan was to reduce the amount of these elements present to the lowest concentration that would allow the production of grain. This concentration would be below that required for the normal development of the plant, and it was thought that in such conditions the effect of the elements on the composition of the plant would be more strikingly brought out. Aside from the greatly reduced calcium or phosphorus content, when the amount of these elements in the respective nutrient solutions was reduced, the most striking results obtained were the low calcium content of the plants of the low phosphorus series and the low nitrogen series, and the high phosphorus content of the plants of the low calcium series and the low nitrogen series. Potassium and magnesium seemed to have little effect on the calcium and phosphorus content of the plant. Climatic factors, on the other hand, were shown to have a decided effect on the composition of the plant. A good bibliography is added.—S. V. EATON.

Alkali soils.—The hardening of irrigated land has been studied by SCOFIELD and HEADLEY,[16] who conclude that neutral salts of sodium as well as the carbonate, "black alkali," may produce hardening of the clay component of soils. The results are about the same, whether sodium-containing water is used on good soils, or whether pure water is used on salty soils; in either case the soil will harden seriously on drying, and become somewhat impervious to water. The theory advanced to explain the action of sodium on clay is that the sodium replaces other bases on the surface of the particles, and becomes in part sodium silicate. This hydrogel coating increases the effective size of each particle, reduces the porosity of the soil, and greatly retards water percolation through it. On drying, the colloidal gel cements the particles together, whence the hardness of these soils. If enough calcium or aluminium is present, however, the harmful action of sodium is obviated. When irrigation water contains more Na and K together than Ca and Mg, it is likely to cause hardening. The Colorado River and its lower tributaries contain too much of the hardening salts. The authors think injury to irrigated land may be avoided by treatment of irrigation water or land with soluble calcium or aluminium salts.—C. A. SHULL.

Nitrogen fixation by green plants.—WANN[17] presents some interesting results of experiments showing that members of the Chlorophyceae can utilize the uncombined nitrogen of the atmosphere. Seven species exhibited this

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[16] SCOFIELD, C. S., and HEADLEY, F. B., Quality of irrigation water in relation to land reclamation. Jour. Agric. Res. **21**: 265–278. 1921.

[17] WANN, F. B., The fixation of free nitrogen by green plants. Amer. Jour. Bot. **8**: 1–29. *pl. 1. fig. 1.* 1921.

power when grown on mineral nutrient agar containing a nitrate and glucose. There was no fixation when nitrogen was supplied in the organic form, and with a nitrate present but no carbohydrate, the amount of fixation was not marked enough to be conclusive. There seemed to be some fixation in the latter case, the lesser amount being due, perhaps, to the much decreased growth where the carbohydrate was omitted. One species seemed to have the power of denitrification as well as nitrogen fixation. The amount of nitrogen fixed by the algal species used compared favorably with the amount recorded by other investigators as fixed by the nitrogen fixing bacteria. The results recorded in this paper are contrary to the generally accepted view as to the ability of green plants to make use of free nitrogen. The possibility of green plants possessing this power of nitrogen fixation, however, is of such great interest both scientifically and economically that the work of WANN should be the stimulus for much more work along this same line.—S. V. EATON.

Variation in stomata and hydathodes.—In a study of the number of stomata per sq. mm. upon leaves of *Campanula rotundifolia* borne upon different parts of the same plant and upon the leaves of plants grown under different conditions of habitat, Miss REA[18] found some interesting variations. In general there was an increase in number from the lower to the higher position of the leaf upon the shoot, and an increase with conditions of increasing dryness. Such increase was least upon the under surface of leaves on different portions of the stem of the same plant. It is suggested that the increased number upon sun shoots compared with those developed in the shade is due to increased photosynthesis, although no causal connection is established. It would be desirable to know the connection between the size of the epidermal cells and the number of stomata, but this information is not given. Groups of hydathodes were found on the upper surfaces of all leaves examined, the number per leaf decreasing from the base to the apex of the shoots.—G. D. FULLER.

Water relations of Pinus and Leucadendron.—Following methods devised by FARMER, the water conducting power of the wood of *Pinus pinaster* and *Leucadendron argenteum* has been measured by AITKEN,[19] and a comparison instituted between transpiration and the rate of water transmission. The rate of transpiration was higher in *Pinus*, both per twig and per unit area, than in *Leucadendron*, as was also the ratio of transpiration to transmission. From the data obtained it would seem that the wood of *Pinus* is capable of transmitting a limited amount of water which it utilizes with a very small margin of surplus.—G. D. FULLER.

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[18] REA, MARGARET W., Stomata and hydathodes in *Campanula rotundifolia* L., and their relation to environment. New Phytol. **20**:56–72. *figs. 6.* 1921.

[19] AITKEN, R. D., The water relations of the pine (*Pinus pinaster*) and the silver tree (*Leucadendron argenteum*). Trans. Roy. Soc. So. Africa **10**:5–19. 1921.

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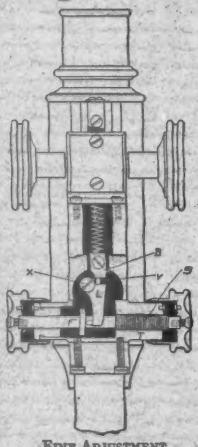

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